ANNALES MEDICINAE EXPERIMENTALIS ET BIOLOGIAE FENNIAE

STUDIES IN HEMAGGLUTININS OF LEGUMINOSAE SEEDS

BY
OLAVI MÄKELÄ

VOL. 35 1957 SUPPLEMENTUM 11

MERCATORIN KIRJAPAINO
HELSINKI, FINLAND

FROM THE DEPARTMENT OF SEROLOGY AND BACTERIOLOGY,
UNIVERSITY OF HELSINKI

STUDIES IN HEMAGGLUTININS OF LEGUMINOSAE SEEDS

BY
OLAVI MÄKELÄ

HELSINKI 1957

Printed by Weilin & Göös 1957

PREFACE

The present work was carried out in 1954 through 1956 at the Department of Serology and Bacteriology, University of Helsinki.

My teacher, Professor K. O. Renkonen, M. D., Director of the Department, suggested the subject of the investigation, which aspires at being a continuation of his studies. At every stage of the work he supported me in very concrete ways. From him I received the bulk of my seed material. He placed at my disposal laboratory facilities and, what is of paramount importance, his knowledge of the subject for me to draw upon. For all this I wish to express my deep gratitude to Professor Renkonen.

I also wish to extend my thanks to Dr. M. Krüpe, M.D., Hygiene-Institut der Universität, Marburg/Lahn, Germany, who has given me valuable advice and who kindly undertook to look this paper through in manuscript.

Dr. H. R. Nevanlinna, M. D., Director of the Finnish Red Cross Blood Transfusion Service, provided me with samples of various kinds of human serum and also offered many invaluable pieces of advice while scrutinising the manuscript. For this I offer him my sincere thanks.

I am also much obliged to Dr. A. E. Mourant, M. A., D. Phil., D. M., M.R.C.P., of the Lister Institute of Preventive Medicine, London, for various valuable test sera, which I used for typing the test cells.

This study would not have been possible without the kind help from the directors of numerous botanical gardens the world over. For this aid I express my heartfelt gratitude. Under particular obligation I am to Mr. L. Ikkala, Chief Gardener, The Botanical Garden, University of Helsinki.

My thanks are also due to Dr. B. Pettersson, Ph. D., who helped me with the botanical problems.

I am greatly indebted to Mrs. Hilkka Kauppi, Mag. Phil., and Miss Eeva Raatikainen, Librarians of the Institute, who spared no pains to make the vast amount of literature available to me.

The manuscript was translated from Finnish by Mr. Matti Salonen, Mag. Phil., to whom I owe a debt of gratitude.

Finally I wish to offer my thanks to my colleagues and many self-sacrificing blood donors. From my wife I have received untiring help all along, which aid I set very special store by.

Helsinki, March 1957

Olavi Mäkelä

CONTENTS

INTRODUCTION

OUTLINES OF THE SEROLOGICAL STUDY OF PLANT AGGLUTININS

For some 70 years plants have been known to contain substances that are capable of clumping or agglutinating homogeneously suspended animal cells. The best known general term for these substances is probably plant agglutinins or phytagglutinins though the designation lectins is known, too (27).

Plant agglutinins were discovered by Stillmark (139) in 1888. Studying ricin, a poisonous substance refined from the seeds of Ricinus communis (Euphorbiaceae), he noticed that it agglutinated the red cells of some animals. These experiments were continued under the direction of Kobert, and agglutinins were thus found in the seeds of other plants, too. Such plants were Croton Tiglium and Abrus precatorius (66, 67).

All the above extracts were poisonous. For this reason the hemagglutinating power of these substances was first regarded as a manifestation of the toxin, it was even suggested that the hemagglutinating power alone accounts for the toxicity. Prolonged differences of opinion existed on the question whether the toxin and the agglutinin were identical until at least a partial separation of the two was achieved (109) and non-toxic plant agglutinins were found (89).

Ricin and abrin (from Abrus precatorius) were found to be good antigens by Ehrlich in 1891 (38). He immunised white mice by feeding them increasing doses of these substances and noticed that immunised mice tolerated e.g. ricin in subcutaneous injections over 200 times as much as control mice. Bacterial toxins and their antitoxins had been discovered at about the same time. Plant agglutinins were now found to be excellent model antigens in the solution of immunological problems. They were much easier to prepare in great quantities than bacterial antigens. They were also much more stable and, what is most important, being both antigens and agglutinins they produced antibodies which lent themselves to study in the test tube.

Several important problems of immunology were solved with the aid of plant agglutinins at the shift of the century. Thus, in 1897, Ehrlich (39) carried out the first quantitative determination of an antibody in vitro by studying the inhibition of ricin agglutination caused by the immune serum. This was possible because the amounts of toxin and agglutinin neutralised by two quantities of antiricin are proportional, by and large.

The first local immunisation was performed by means of abrin in 1901. Römer (126) applied this substance to the conjunctiva of rabbits. When he dropped this substance onto one conjunctiva only, immunity developed in that conjunctiva but not in the other.

Also the so called Danysz's phenomenon, i.e. that a given quantity of an antibody neutralises less toxin if the latter is added gradually during 24 hours than if it were added at once, was originally demonstrated by means of the ricin-antiricin system (33).

One of Landsteiner's (87) first tests concerning the reversibility of the antigen-antibody reaction was done by means of red cells that had been agglutinated by abrin. After the cells had been washed, they were placed in an incubator at 50° suspended in saline solution. After the removal of the cells from this solution it agglutinated strongly.

Gradually, however, the preparation and study of bacterial antigens became more efficient thus making it possible to dispense with model antigens. With the coming of the twenties the attention paid to plant agglutinins was on the decrease, and Ford's (49) statement made in 1913 that «interest in the study of this group of poisons is constantly increasing, however, and the next few years are likely to see more complete and elaborate investigations and results of the most far-reaching importance» did not prove exactly correct.

Stillmark (139) already observed that ricin agglutinated the red cells of different animal species somewhat selectively. Most of the agglutinins discovered later on also showed such a slight degree of selectivity. This fact did not pass unnoticed and it was realised that an identification of the blood of different animal species might be facilitated by it.

As far as the author knows, before 1947 only two positive attempts had been made at finding blood group specific plant agglutinins. Marcusson-Begun (103) studied the tubers of numerous potato strains and found a substance in them, which agglutinated the red cells of all the mammalian species examined. Sievers (133) studied «brown beans and green peas» against human red cells. Neither found marked differences between the titres of the several people studied.

A third such test was conducted by Renkonen (122) during 1947 and 1948. Whereas earlier studies had involved only one or two species, Renkonen's series comprised the seeds of 99 species, all of the family Leguminosae. Six of the seed extracts showed definite affinity either for A or for 0 red cells.

As a result of this finding the study of plant agglutinins was enhanced once more. The following years witnessed numerous investigations of extensive seed materials so that by the year 1955 the total number of species studied was estimated at one thousand (115). Species that contain agglutinins exhibiting different degrees of blood group specificity were discovered; of these an overwhelming part belonged to the family Leguminosae.

The botanical nature of agglutinins and their function in the plants have not as yet been explained beyond pure conjecture. The treatment of this subject will be found in Chapter II.

CHEMISTRY OF PLANT AGGLUTININS

The chemical nature of the seed agglutinins has been subjected to the most extensive studies. Stillmark (139) already concluded that ricin was a protein. Later on, however, the agglutinin of ricin turned out to be strongly resistant to the action of proteolytic enzymes (109). Since similar observations had been made in the study of other plant agglutinins (147), too, it was also suggested, at the beginning of the century, that agglutinins might not be proteins.

A couple of subsequent investigations oppose the protein theory. In 1949 Bourdillon (18, 19) reported that he had refined the agglutinins of bean and influenza virus by quite similar

methods securing from both a highly active substance soluble in organic solvents. Accordingly, in his opinion the agglutinin of bean is not a protein. In 1953 Cugudda et coll. (32) put forward still another suggestion concerning the structure of plant agglutinins. They had analysed the substances of Vicia faba seeds and consider the glycoside vicine to be the most powerful agglutinant. It is true, they think it possible that impurities in their glycoside might account for the agglutination. Neither of these theories has been proved correct as far as the author knows. The majority of workers at present regard agglutinins either as proteins or as mucoproteins.

Landsteiner and Raubitschek (89) regarded the plant agglutinins they had found as proteins. They succeeded in refining the agglutinins by methods showing elective affinity for proteins, such as precipitation by alcohol, ammonium sulfate and slight acidification. Russ and Oesterlin (130) employed the same methods for refining the agglutinins of Soja hispida and Phaseolus vulgaris. They precipitated the impurities by means of 20—30 % $(NH_4)_2SO_4$ saturation and the agglutinins by means of 50 % saturation. Fujiwara (53) refined the agglutinins of soy bean by adsorbing them onto kaolin, aluminium hydroxide or calcium phosphate followed by elution in weak alkalis.

From the seeds of Canavalia ensiformis Sumner and Howell (140) refined a crystalline globulin called concanavalin A, which agglutinated the red cells of horse at the final dilution of 1:10 000 000. The crystals were found to be isoelectric at pH 5.5. It was also noticed that 0.1 N. NaOH and HCl destroy the agglutinins, reversibly at first, irreversibly later.

In 1950 Renkonen (123) conducted a chemical investigation which included the blood group specific agglutinin of Vicia cracca and the agglutinin of Phaseolus vulgaris. The seed extract was prepared in 0.9 % NaCl solution. The precipitation of impurities was effected with 20 % alcohol concentration, that of the agglutinins from the supernatant with acetone. The precipitate dissolved in NaCl was treated with chloroform, and the acetone-chloroform treatment was still repeated twice. This procedure yielded highly active preparations, which contained approximately 30 % carbohydrates and were quite homogeneous electrophoretically. Renkonen suggested the

possibility of the agglutinins being mucoproteins. Using this same technique Koulumies (71) refined the agglutinins of Cytisus sessilifolius. Both a Vicia cracca preparation refined by the Renkonen technique and a crude extract dialysed against dextran were subjected to electrophoretic investigation by Krüpe (84). He was surprised to find that both contained only one homogeneous protein fraction that migrated in the manner of globulins.

Rigas and Osgood (125) refined the agglutinins of Phaseolus vulgaris by using fractioning alcohol precipitation followed by fractioning ammonium sulfate precipitation. They obtained a preparation the activity of which was comparable to Renkonen's Phaseolus preparation, and which they found to be a mucoprotein. At a low pH it was hydrolysable into an inactive polysaccharide and a hemagglutinating euglobulin. This protein hemagglutinin was approximately 100 times as active as the mucoprotein agglutinin. In the opinion of the authors the increase in activity is not accounted for by a decrease in the amount of inactive substance, instead they think it possible «that the elimination of the polysaccharide unveils more active sites in the protein which may have been sterically hindered or which may be participating in the bonds with the polysaccharide».

Boyd (23) showed that a pressure of 6000 atmospheres destroys the dissolved agglutinins of Phaseolus limensis. He also coupled various diazotised amines to the Lima bean globulin and found that a considerable amount of these could be coupled without an appreciable decrease in the agglutinating power of the globulin taking place. Hence he inferred that the amino groups of the globulin molecule are not essential to the agglutination, even though the agglutinins are destroyed by formaldehyde treatment (22).

Liener and Wada (96) modified a purified soy bean hemagglutinin by acetylating its amino groups. They found that the modification of the easily acetylated α-amino groups brought about no significant loss of the agglutinating power. The acetylation of the ε-amino groups in higher acetic anhydride concentrations was accompanied by a considerable decrease in the hemagglutinating activity. They also showed the essentiality

of phenolic groups in agglutination. On the other hand, the removal of the C-terminal amino acids by the action of carboxypolypeptidases was accomplished without loss of activity.

The greater thermoresistance of human incomplete agglutinins compared with the complete is known (106). Incomplete agglutinins have been found also in plant extracts. No appreciable differences seem to exist in the sensitivity to heat between the complete and incomplete plant agglutinins (101).

Studies in the chemistry of plant agglutinins outside the seeds are few. According to Marcusson-Begun (104) the agglutinins of potato tubers do not withstand boiling in the least. According to Bird (11) they withstand boiling for 30 minutes. After having carried out fractioning precipitations by ammonium sulfate, Krüpe (84) adopted the view that the agglutinins of potato tubers as well as those of seeds are globulins.

OBJECT OF THE PRESENT INVESTIGATION

The aim of this study was

1. To throw light on the effect of physical factors on the agglutination by seed extracts.

2. To obtain information about the modes of reaction of incomplete seed agglutinins when tested by different methods, and to study the incidence of such agglutinins.

3. To study the botanical rules that possibly govern the occurrence of seed agglutinins in the family Leguminosae.

4. To discover new and possibly more useful reagents for the use of the routine blood group serologist.

5. To obtain information of the homogeneity of seed agglutinins, whether prosthetic groups of only one kind or of several kinds are contained in a given extract; and if there are several, whether these are parts of one molecule or of different molecules.

6. By means of agglutination-inhibition tests to study the structure of those red cell receptors with which the seed agglutinins react.

I SEROLOGICAL ACTIVITY OF PLANT EXTRACTS

SURVEY OF LITERATURE

1. AGGLUTINATION

Direct agglutination of red cells

In most investigations into plant agglutinins the red cells have been suspended in physiological NaCl solution. The agglutination thus achieved resembles, both macroscopically and microscopically, that caused by animal agglutinins. The agglutinins can be absorbed with the red cells though apparently not so easily as animal agglutinins. Strangely enough, originally inactive extracts have sometimes been found to agglutinate human red cells, after absorption of the extract with these cells (84). This phenomenon has been explained by assuming that the cells absorb «inhibiting agents», maybe blocking antibodies, which results in the activation of the extract. Accordingly, in the opinion of Krüpe (84), results from the absorption tests of plant agglutinins are to be evaluated with caution.

Elution of agglutinins from the treated cells can be effected e.g. by treatment with heat at 50°—56° (87). On the other hand, spontaneous elution does not take place at 37°, and the red cells from which the agglutinins are set free can be agglutinated once more with the same agglutinin (84).

Indirect agglutination of red cells

Incomplete animal agglutinins have been known for many years (57). They are commonly believed to occur mainly in immune sera. Therefore it was with a certain degree of surprise that, in 1954, Krüpe (81) found some extracts from the seeds of Sophora japonica incapable of agglutinating the red cells of the B group in saline milieu while agglutinating in AB serum or bovine albumin milieu. Plant agglutinins, of course, could hardly be regarded as immune antibodies.

The behaviour of the incomplete plant agglutinins was similar to that of the animal agglutinins in many respects. They agglutinated enzyme treated red cells in saline milieu, and they could be used to elicit the phenomenon of Coombs, Mourant and Race by using anti-seed-protein immune serum (82).

Krüpe (82) succeeded in preparing Sophora extracts containing complete agglutinins, too. Such extracts, particularly, as were made taking great care to remove the scales agglutinated in saline milieu. Moreover, Krüpe noticed that extracts which were kept at room temperature developed saline agglutinins spontaneously within 1—3 weeks. Further he was able to develop saline agglutinins in the extract by absorbing it with carbon or red cells. For this reason he suggested that during the extraction such substances are dissolved from the scales of the seeds as inhibit saline agglutinins. Afterwards these substances supposedly disappear at room temperature through (fermentative ?) decomposition, or are absorbed by carbon or red cells. The present author developed saline Sophora agglutinins according to Krüpe's method by aging (101).

Subsequent investigations have demonstrated incomplete agglutinins in several blood group specific plant extracts at higher dilutions than saline agglutinins (14, 34, 84). On the other hand, very few such extracts have been discovered as contain incomplete agglutinins only.

Bird (16) reports that Glycine soja extract, which is known to contain a complete anti-rabbit agglutinin (84) and a cold agglutinin of the complete type against human red cells (9), agglutinates papainised human red cells or those suspended in bovine albumin, also at 37°. His absorption and elution tests indicate that all these diverse reactions are caused by one and the same agglutinin. McNeil et coll. (104) report that Lotus tetragonolobus extract acts as a good anti-H reagent in 10 % polyvinylpyrrolidon milieu.

Phenomena evidently caused by incomplete agglutinins were recorded by investigators of plant agglutinins at the beginning of the century already, though it was not until Krüpe's discovery that a common explanation was provided. In 1909 Miessner and Rewald (105) noticed that while the serum of most animals inhibits ricin agglutination it is prompted by the serum of cow.

A somewhat similar finding was made by di Macco (98) in 1923. Friedemann (51) treated red cells with a dose of ricin insufficient to agglutinate them whereupon he transferred the cells into antiricin capable of inhibiting agglutination under normal circumstances. In this way he brought about an agglutination by anti-globulin method. Craeger and Gifford (31) found that 10 % gum acacia had a promoting effect on the agglutination by Vicia faba extract.

Agglutination of other corpuscles

Stillmark (139) was the first to note the agglutination by ricin also of liver cells, epithelium cells and leucocytes, and Wienhaus (147) managed to agglutinate liver cells with his Phaseolus extract. Rosenthal (129) found that the Phaseolus multiflorus extract agglutinated leucocytes, platelets, spermatozoids, yeast cells, and spores of molds. The solutions used by all these scientists were apparently rich in agglutinins. The agglutinability of leucocytes, at least, is obviously lower than that of red cells, since it has been possible to isolate the former from blood and bone marrow by agglutinating the red cells away with Phaseolus extract (95, 134). It has also been found possible to agglutinate reticulocytes by means of suitable agglutinin concentrations while nucleated erythrocytes remain homogeneously suspended.

Contradictory findings are reported on the phytagglutination, the agglutination by plant extracts, of bacteria. Kritschewsky (74) reported an agglutination of typhoid bacilli and cholera vibrios by juice squeezed from the leaves of Echeveria Scheideckeri (Crassulaceae). Marshal (102) observed that juice squeezed from the leaves of Echeveria gibbiflora agglutinated B. prodigiosus and Proteus X 19. He found, however, that proteins precipitated from the juice with ammonium sulfate were inactive, and the clumping was probably due to tannin present in the juice. Krüpe (84) has ascertained the juice of Echeveria Scheideckeri inactive, and that an unspecific clumping of bacteria and erythrocytes is caused by the tannin.

Eisler and Portheim (42) reported that they had achieved a weak agglutination of typhoid bacilli and cholera vibrios by

means of the seed extract of Phaseolus vulgaris. Sumner and Howell (140) noticed that a solution of concanavalin A (a crystalline globulin from Canavalia ensiformis seeds) agglutinates mycobacteria and actinomycetes. Isotalo (58) studied the effect of the seed extracts from 40 Leguminosae species on 21 different species of bacteria. She did not observe a single case of unmistakable agglutination. Krüpe (84) arrived at a similar result upon studying 131 plant extracts, which did not contain hemagglutinins, and 19 bacterial species, against one another. Nor did the anti-A and anti-H extracts studied by him agglutinate a single pneumococcus strain of the twelve examined, not even pneumococcus XIV. They did not bind hemagglutinins either, nor was capsular swelling observed.

Schmidt (132) reports that the Vi-antigen of S. typhi, S. paratyphi and certain coli strains bind the anti-H of both bovine serum and Laburnum alpinum. Punin (118) observed the agglutination of Bacillus botulinus and Bacillus histolyticus by Laburnum alpinum extract.

Influence of physical and chemical factors

Most plant agglutinins, both complete and incomplete, react almost independently of the temperature within the range of 4°—40° C (84). Elo and Estola (46), it is true, found that a fresh extract from the fungus Marasmius oreades reacted more strongly and specifically at 37° than it did at lower temperatures. Krüpe (84), however, thinks that the same result can be achieved by hot treatment of the extract prior to the agglutination. In the extracts of Glycine soja, Bird (9) has detected an agglutinin reactive with human red cells in saline milieu at low temperatures (+4°) only.

As already stated, elution of plant agglutinins from cells is possible at higher temperatures (50°—56°).

Actual tests have shown that plant agglutinins do not agglutinate red cells in a milieu that is poor in electrolytes. This was known to Rona and György (127) as early as 1920, and Furihata (54) came to the same conclusion. Krüpe (84) noticed that the anti-A of Vicia cracca does not agglutinate A-cells, nay,

does not even become attached to the cells, in a milieu poor in electrolytes.

The dependence of phytagglutination on pH has not been much studied. Sumner and Howell (140) found an agglutination of red cells by crystalline concanavalin A taking place within a pH range of 5.2—7.5. Rosenthal (129) reports that Phaseolus multiflorus extract agglutinates at pH 3—12.

2. ANTIGENICITY

Reference has already been made to the great importance of the vegetable antigens in the early history of general immunology. Since they were superseded by other antigens in this field, they have interested blood group serologists.

Ehrlich (39) was the first to demonstrate the inhibition of ricin agglutination by anti-ricin. A problem of serological interest has been whether an actual anti-antibody has thus been created, i.e. whether it is mainly the «prosthetic group» of the plant agglutinin or the plant agglutinin molecule in general that has acted as an antigen. In order to make this matter clear both Renkonen (123) and Krüpe & Powilleit (76) have studied the agglutination-inhibiting power of immune sera that have been made by means of blood group specific plant extracts. The immune sera obtained in this way readily neutralise their homologous antigen (plant anti-A) but fail to neutralise anti-A isoagglutinin. Accordingly it seems that only anti-plant protein sera were produced.

3. PRECIPITATION

Stillmark (139) already noticed that mixtures of ricin and normal serum grew turbid. Kraus (72) examined this phenomenon more thoroughly and came to the conclusion that a given plant extract precipitates the sera of those animal species only whose cells it agglutinates. He was of the opinion that one and the same agent brings about both the precipitation and the agglutination. Kraus's theory was supported by Stillmark's (139) observation of the moderate inhibition of ricin agglutination by normal serum. The findings of Raubitschek and Wilenko (120) spoke against Kraus's theory. They noted that pea extract did

not agglutinate the red cells of chicken but it precipitated with the serum; moreover, when the extract was mixed with the serum of rabbit and the resultant deposit removed, the supernatant agglutinated but did not precipitate. Sumner and Howell (140) also found that concanavalin A caused precipitation in the sera of all studied animal species, though it did not agglutinate the red cells of every species. Wilenko (148) noticed that precipitation occurred when different plant extracts were mixed.

Jacoby (60) has found that in homologous immune sera the precipitation caused by plant extracts is more intense than in normal sera. Dujarric de la Rivière et al. (35) have shown that an immune serum produced by means of pea extract causes precipitation in bean extract, too, but the precipitation lines obtained by the gelatin plate technique are different. In this way it has been possible to distinguish between different varieties of a plant species.

The precipitation of blood group substances with plant extracts is treated in Chapter III.

4. HEMOLYSIS AND OTHER REACTIONS

Many plant extracts have been found to hemolyse red cells (67, 122, 20). This is particularly true of numerous fungous extracts (50). Ford (49) was able to absorb the hemolysin of Amanita muscaria by red cells at a temperature of approx. 0° C. Hemolysis did not take place in cold state but it occurred as soon as the cells were separated from the extract and transferred to warm environment. After that the absorbed extract did not hemolyse any more, even when warm. This indicated that the hemolysis was not due to physical factors, e.g. to pH. That the same applies also to ricin hemolysis was shown by v. Eisler (41); subsequently it has turned out to be true of other seed extracts, too (20). Ehrlich (40) suggested that the hemolysin and agglutinin of ricin and abrin are identical and the phenomena differ only quantitatively.

Nungester and van Halsema (112) have found that particular Phaseolus vulgaris extract, which agglutinates the leucocytes of rat, to inactivate the Flexner-Jobling carcinoma cells of rat,

so much so that they do not produce cancer when implanted in rats. Four other Phaseolus extracts did neither agglutinate leucocytes nor inactivate cancer cells.

The seeds of Vicia faba and some other Leguminosae species are known to partake in the etiology of certain cases of hemolytic anemia of man. Investigations have been carried out concerning the possible share of this seed agglutinin in the causation of the malady (31, 32, 48). No evidence to such an effect probably exists at present. It has been found that plant agglutinins do not hemolyse red cells in the presence of complement (31, 28, 84).

OWN INVESTIGATIONS

I. METHODS

Since preliminary tests are described in this connexion, which were largely done in order to discover suitable methods, the material and methods will be discussed again later on.

The seeds were ground in a mortar. The powder (1 unit of weight) was mixed with physiological salt solution (9 units of weight), and the mixture was allowed to remain at 37° C for two hours. During this period it was stirred once. After two hours the extract was centrifuged (10 mins at 3000 r.p.m.) and the resulting opalescent supernatant was used as such in the agglutination tests. In general, fresh extract was used, exceptionally, however, the extract had been kept at —18°C for 0—4 months prior to use. No change in the agglutination titre was found to have happened during this time, unless the extract was melted in the meantime.

Red cells washed from citrated blood were used as test cells. As a rule they had been taken on the same day, sometimes kept for 18—70 hours in the original plasma at +4°C. The titres obtained with plant agglutinins against such preserved red cells were found to be nearly identical with those obtained against the fresh red cells of the same person.

Unless stated otherwise, the technique used was as follows. Twofold serial dilutions in saline were prepared from the extracts. The red cells were used in a 2 % saline suspension. Agglutination was allowed to take place in glass test tubes (10×100 mm). One drop of plant extract and two drops of the cell suspension were put into the tubes using a Pasteur pipette (diam. of opening 1.5 mm). The tubes were allowed to remain for 1.5—2 hours at room temperature. Such variations of the incubation time were found to have a negligible effect on the titre. The agglutinations were read with the unaided eye and with a low power microscope. A distinct agglutination was considered accomplished when the larger part of the red cells had been collected into clumps. The denominator of the final dilution of the seed extract causing a distinct agglutination is given as the titre.

Effect of temperature

Six seed extracts showing different blood group specificity were tested at various temperatures in saline and serum (the test cells suspended in human AB serum) milieu.

TABLE I

Agglutination titres of seed extracts against different kinds of red cells at various temperatures

milieu	cells	temp.	Extract of seeds of					
			Phaseolus vulgaris	Vicia cracca	Dolichos biflorus	Phaseolus lunatus	Sophora japonica	Laburnum Watereri
saline	A_1	4°	1024	4096	32	256	—	—
		18°	1024	2048	128	512	—	—
		37°	2048	4096	256	512	—	—
	B	4°	2048	8	—	8	—	4
		18°	1024	8	—	4	—	4
		37°	2048	2	—	—	—	—
	O	4°	1024	8	—	—	—	16
		18°	2048	4	—	—	—	16
		37°	2048	2	—	—	—	8
AB serum	A_1	4°	2048	32768	64	4096	256	2
		18°	1024	16384	512	4096	128	2
		37°	4096	32768	1024	8192	64	—
	B	4°	1024	64	—	2	2048	16
		18°	1024	64	—	—	1024	16
		37°	2048	32	—	—	512	4
	O	4°	2048	64	—	—	4	64
		18°	2048	64	—	—	4	64
		37°	2048	32	—	—	2	16

The results are given in Table 1. As the table shows, the effect of temperature within the studied range (+4°—+37°) is small. The agglutinins of Sophora japonica and Laburnum Watereri react a little more strongly at a low temperature. The anti-A agglutination by Phaseolus lunatus becomes more

marked at the higher temperatures, whereas its reaction with B cells is strongest at a low temperature. The effect of temperature on saline agglutination seems to be about equal to that on indirect agglutination.

Effect of electrolytes

Some seed extracts were dialysed for 24 hours against running water in order to determine the effect of NaCl concentration on phytagglutination. After this their electrolyte concentration was less than 0.005 moles per litre measured by conductivity. During the dialysis unknown substances were precipitated from the extracts, the volume of which increased a little at the same time, while the decrease in agglutinin content varied in different extracts.

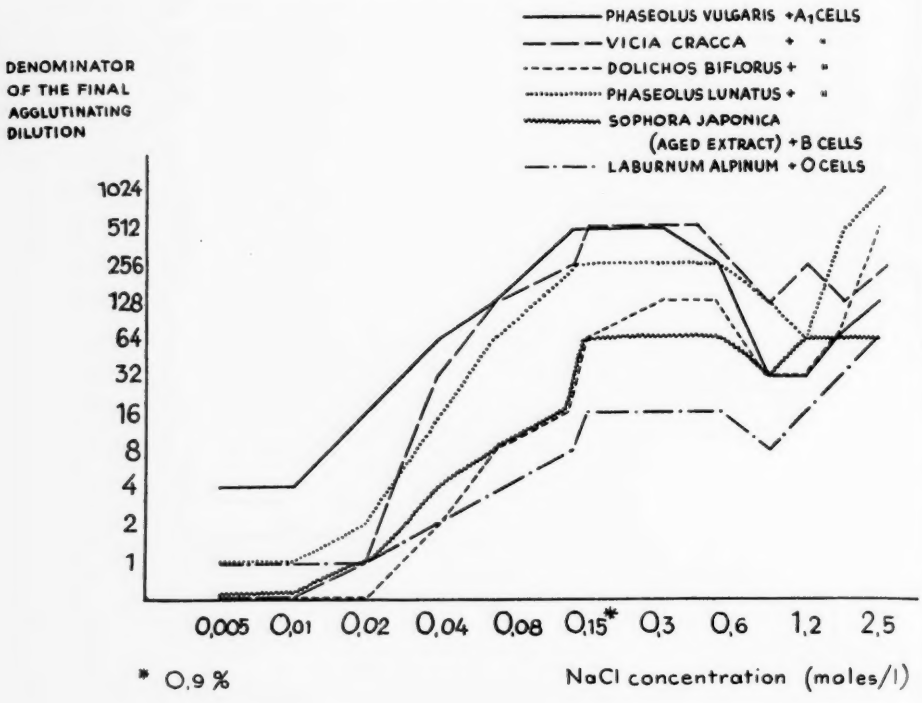
Tests with hypotonic salt concentrations were conducted as follows. The dilutions of the extract were prepared in distilled water. The red cells used were suspended in solutions whose NaCl concentration varied from 0 to 1.8 % and to which enough glucose was added to make the osmotic pressure of the solution the same as that of a 0.3 M (1.8 %) NaCl solution. When equal volumes of seed extract and the cell suspension were mixed in agglutination-titration, the osmotic value of the mixture was the same as that of physiological salt solution and the salt concentration one half of that of the cell suspension. Glucose in 5 % concentration was not found to inhibit the agglutination caused by the seed extracts here examined if the NaCl concentration of the milieu was 0.9 %.

When studying the effect of hypertonic salt concentrations on agglutination, the dialysed seed extracts were used. The dilutions were made in 0.15—2.6 M NaCl solutions. The test cells were suspended in NaCl solutions of identical concentrations. The seed extract and the cell suspension were mixed in equal volumes.

In solutions of very low electrolyte concentration (less than 0.005 moles per litre) the red cells had a spontaneous tendency to clump into aggregates that did not resemble agglutination clumps, but otherwise the cells were homogeneously suspended in control tubes. Cells that had been suspended in a 30 % NaCl solution were quickly hemolysed, while those suspended in a 15 % (2.6 M) solution showed no definite signs of damage.

The results are given in Fig. 1. It will be seen that a decrease in the NaCl concentration below the physiological molarity 0.15 is generally accompanied by a considerable fall in the titres. Extracts that agglutinate still in a 0.005 M salt concentration exist, however.

Fig. 1. — Agglutination titres of seed extracts in various NaCl concentrations



At higher salt concentrations the agglutination seems to be somewhat irregular. With an increase in the concentration from 0.15 moles per litre up to a molarity 0.8 there is a slight fall in the titre. At still higher salt concentrations a rise in the titre is often observed. Nevertheless, the controls (salt solution + cell suspension) were totally negative also in these tests, and anti-A extracts from Dolichos biflorus and Phaseolus lunatus retained their specificity even at 2.6 M salt concentration.

In order to study the effect of different kinds of electrolytes on phyt-agglutination 0.3 M solutions of NaCl, KCl and $NaNO_3$ as well as 0.2 M solutions of $MgCl_2$, $CaCl_2$, $BaCl_2$, Na_2SO_4 and Na_2HPO_4 were prepared. The red cells were suspended in these, and the plant extracts poor in electrolytes were used for the agglutination. The seed extract and the cell suspension were mixed in equal volumes.

The results are given in Table 2. The solutions of the salts

TABLE 2

Agglutination titres of seed extracts in the presence of various electrolytes in 0.15 M concentrations

The salt present	Extract of seeds of				
	Phaseolus vulgaris	Vicia cracca	Dolichos biflorus	Phaseolus lunatus	Laburnum alpinum
	tested with red cells of blood group				
	A_1	A_1	A_1	A_1	O
NaCl	512	512	64	256	16
KCl	512	256	64	128	16
$MgCl_2$	512	512	256	512	32
$CaCl_2$	512	512	512	512	32
$BaCl_2$	512	512	512	512	32
$NaN0_3$	512	512	64	256	16
Na_2SO_4	512	512	256	512	32
Na_2HPO_4	512	512	32	256	16

examined showed no marked differences in their effects on agglutination.

Effect of pH

The effects of variations in pH on the agglutination were studied within the approximate range of pH 4.5—pH 11, where red cells are not hemolysed. The solutions were buffered

at pH 4 — pH 6 with a 0.15 M acetic acid — Na-acetate mixture,
at pH 5.5 — pH 8.5 with a 0.12 M NaH_2PO_4—0.1 M Na_2HPO_4 mixture
and at pH 8.5 — pH 11.5 with a 0.1 M Na_2HPO_4—0.15 M NaOH mixture.

The red cells used were suspended in undiluted buffer solutions. One drop of seed extract and two drops of cell suspension was put into the agglutination tubes. In order that an excess of buffering protein might not come into the solution, more dilute crude extracts than usual were used. The final agglutination mixtures consisting of the plant extract and buffered red cell suspension were also made in greater quantities and their pH values were read in order to control the potency of the buffer. It was found that the readings obtained could be lower by 0.4 unit at the highest pH values than the pH of the mere buffer. No changes to speak of had taken place at the pH values 4—10 as a result of the additional red cells and plant agglutinin. The pH values of the final mixtures were chosen when giving the results.

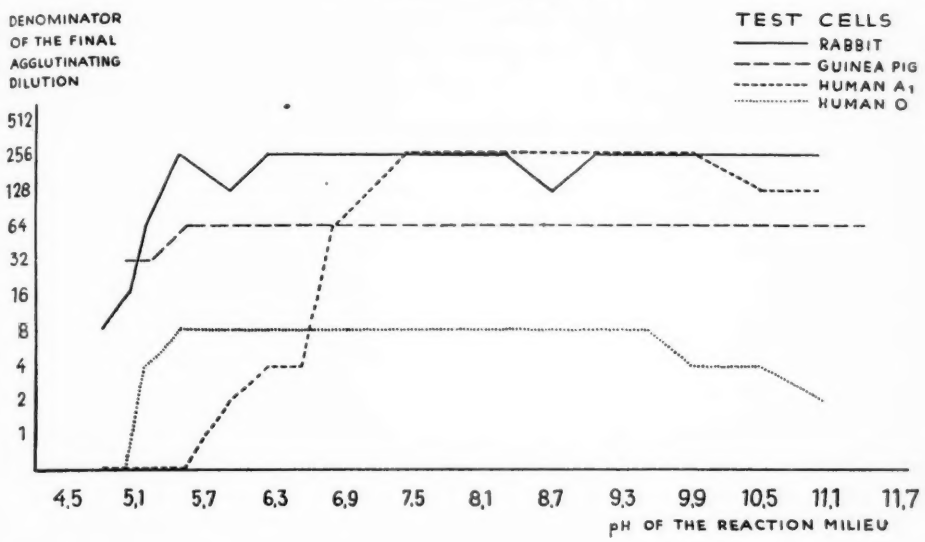
TABLE 3. Agglutination titres of seed extracts in a medium of varying pH

pH	Extract of seeds of											
	Vicia ervilia	Vicia cracca				Vicia villosa	Dolichos biflorus	Phaseolus lunatus	Sophora japonica [1]	Bandeiraea simplicifolia	Laburnum Watereri	Tetragonolobus purpureus
	tested with the red cells of											
	A_1	O	rabbit	guinea-pig	A_1	A_1	A_1	A_1	B	B	O	O
4.3	h	h	h	h	h	h	h	h	h	h	h	h
4.5	h	h	h	h	h	h	h	h	1	h	h	h
4.8	—	h	8	h	—	—	—	128	2	—	2	8
5.0	—	—	16	32	—	—	1	128	1	—	8	8
5.2	—	4	64	32	—	—	8	128	2	—	8	8
5.5	1	8	256	64	—	1	8	128	4	—	8	8
5.9	4	8	128	64	2	2	16	128	4	1	8	8
6.2	4	8	256	64	4	4	8	128	4	2	8	8
6.5	8	8	256	64	4	8	8	128	4	4	8	8
6.8	16	8	256	64	64	32	16	128	4	8	8	8
7.1	16	8	256	64	128	512	8	128	8	8	8	8
7.4	16	8	256	64	256	512	8	128	4	8	8	8
7.7	16	8	256	64	256	512	8	128	4	16	8	8
8.0	16	8	256	64	256	512	8	128	4	8	8	8
8.3	16	8	256	64	256	512	8	128	4	8	8	8
8-7	16	8	128	64	256	512	8	128	4	8	8	8
9.1	8	8	256	64	256	512	8	128	4	8	8	8
9.5	4	8	256	64	256	512	8	128	4	8	8	4
9.9	4	4	256	64	256	512	8	128	4	8	8	2
10.5	2	4	256	64	128	64	4	64	4	8	4	—
11.0	1	2	256	64	128	8	2	16	4	4	1	—
11.4	h	h	h	64	h	h	h	1 (h)	4 (h)	2 (h)	h	h
unbuffered extracts (pH 6—7)	16	8	256	64	256	512	16	128	8	16	8	8

[1] aged extract

Fig. DEN OF AGG DIL 512 256 128 64 32 16 8 4 2

Fig. 2. — Agglutination titres of Vicia cracca extract against different kinds of red cells in a medium of varying pH



The anions CH_3COO^-, $H_2PO_4^-$, HPO_4^{--} and Cl^- were found to be nearly equivalent with regard to the agglutination. The results were read after 1 hour with the naked eye.

The results are given in Table 3 and Fig. 2. It was found that most seed extracts agglutinate within the wide limits of pH 5 and pH 11, more weakly, it is true, near the extremes, which is perhaps due, partly at least, to damage to the cells. There are agglutinins, however, whose titre is practically constant, as long as the cells are whole, e.g. the anti-guinea pig agglutinin of Vicia cracca. There are a few extracts the titre of which slopes down gently at rather low pH values, viz. Vicia ervilia, Dolichos biflorus and Bandeiraea simplicifolia.

The behaviour of the anti-A agglutinin of Vicia cracca and Vicia villosa is of interest. These show a sharp fall of the titre at the transition from pH 7.1 to pH 6.4. The Vicia ervilia extract, which contains no anti-A but an unspecific anti-man agglutinin, was also tested with A_1 cells and it reacted differently.

When the extract of Vicia cracca was buffered at pH 5.0, a slight precipitate formed in 2 hours. This was removed by centrifuging for 15 mins at 4000 r.p.m. and the supernatant

adjusted to pH 7.5. Its agglutination titre was found to be practically the same as that of the untreated extract. If a tube containing agglutinated cells was adjusted again to pH 5.0, a breakdown of the aggregates followed.

A_1 cells that had been saturated with Vicia cracca agglutinin at pH 7.5 were washed three times and suspended in a buffer of pH 5.0. After the aggregates had broken down, the suspension was centrifuged and the supernatant adjusted to pH 7.5. The agglutination titres of this liquid (a kind of eluate) against A_1 and guinea pig cells corresponded with one another like the titres of the original extract, respectively. Judging by the foregoing it seems that the anti-A agglutinin of Vicia cracca is not destroyed at pH 5.0; instead, it appears that the agglutinin is not at all or easily coupled to the red cells at this pH.

3. COMPARISON OF DIFFERENT METHODS FOR DETECTING INCOMPLETE PLANT AGGLUTININS

Hummel (57) divides antibodies according as their degree of incompleteness varies. He says that «aggloids» are demonstrable by means of colloid-conglutination tests only, «cryptagglutinoids» with the aid of enzyme treated cells, too, while less incomplete «agglutinoids» agglutinate intact cells in a serum or albumin milieu.

The present author has studied the hemagglutination caused by such extracts of Sophora japonica and Coronilla varia as did not react at all with intact red cells in saline milieu, accordingly containing incomplete agglutinins only.

The seed extracts were prepared as above and they were diluted in saline. The following additional solutions were used in the test:
Serum, inactivated human AB serum
Albumin, bovine (Armour)
Polyvinylpyrrolidon, Kollidon, biologically examined, molecular weight 28 000 (BASF)

The enzyme treated cells were prepared as follows (86): Papain stock solution was prepared by dissolving 1 g of papain in 100 ml of 0.9 % saline. It was kept for a few months at —18° divided into small tubefuls, which were melted for use, one at a time.

Papain working solution was prepared immediately before use by mixing 1 part of papain stock solution and 9 parts of phosphate buffered saline (1 part Sørensen phosphate buffer pH 7.3 and 9 parts 0.9 % saline).

To 1 ml of packed, thrice washed red cells was added 2 ml of the 0.1 % papain working solution. The suspension was incubated for 30 minutes in water bath at 37° with intermittent agitation by shaking. After this time the suspension was centrifuged, the supernatant removed and the cells were washed three times. New papain treated cells were prepared each day.

Different concentrations of AB serum, albumin and kollidon were used, diluted in physiological NaCl solution. The red cells were suspended in these solutions or in 0.9 % saline. In the agglutination tests one drop of the seed extract and two drops of the cell suspension were added to the tubes. Thus the final concentration of AB serum, albumin and kollidon was 2/3 times their concentration in the original red cell suspension.

The results are given in Table 4. It was found that kollidon could not be used nearly so strong, or in a 7 % solution, as recommended by Hummel (57). Even fresh cells produced rouleaux formations in it or became brown and poorly agglutinable. If the cells had been kept in a refrigerator overnight, a 5 % solution was enough to cause such behaviour; at times the cells, though fresh, did not stand even the 5 % solution of kollidon.

The two agglutinins studied produce almost the strongest reaction in AB serum milieu, agglutination is distinct in a milieu containing as little as 17 % serum. The extracts agglutinate papainised cells rather weakly, and prozone phenomenon is observed. Kollidon does not seem to be a particularly good milieu if incomplete plant agglutinins are to be found.

DISCUSSION

As regards the effect of temperature on phytagglutination, the findings of Krüpe (84) are corroborated by the present author's tests. Krüpe noticed that the phenomenon showed considerable independence of temperature. This seems to be true also of the incomplete plant agglutinins, which is remarkable since many animal incomplete agglutinins react poorly at low temperatures. The present author did not find cold agglutinins in seeds, not even among his Glycine soja samples (See Ch. II), as Bird (9) succeeded in doing.

On the ground of his investigations the present author cannot wholly agree with Krüpe's view (84) that for a visible hemagglutination to take place a certain salt concentration is necessary.

Titres of the incomplete agglutinins of Sophora japonica a

Titre 1/	Sophora japonica test cells suspended in										
	NaCl	AB serum			albumin			kollidon			papain treated cells in saline
	0.9%	25%	50%	100%	5%	10%	20%	2.5%	3.6%	5%	
1	—	++	+++	+++	++	+++	+++	+++	+++	++	+
2	—	+++	+++	+++	++	+++	+++	++	+++	—	++
4	—	+++	+++	+++	+	+++	+++	—	+++	—	++
8	—	+++	+++	+++	—	+++	+++	—	—	—	++
16	—	++	+++	+++	—	+	+++	—	—	—	+
32	—	+	++	+++	—	—	+++	—	—	—	+
64	—	+	++	+++	—	—	+	—	—	—	—
128	—	—	+	+++	—	—	—	—	—	—	—
256	—	—	—	++	—	—	—	—	—	—	—
512	—	—	—	+	—	—	—	—	—	—	—
1024	—	—	—	—	—	—	—	—	—	—	—

\+ More than half of the cells are gathered into aggregates, part of which are discernible to the naked eye
++ Nearly all cells gathered into aggregates
+++ No unagglutinated cells, only few large lumps

Apart from this the author's tests confirm Krüpe's observation that the crude extract from Vicia cracca seeds does not agglutinate A cells in low salt concentrations. Some other extracts, e.g. Phaseolus vulgaris, however, produce an unmistakable agglutination still in 0.005 M NaCl solution. True, a decrease in the salt concentration is accompanied by a fall in the titre also in these cases. A visible agglutination is elicited in solutions that are still poorer in salt, but in these the readings are not reliable in the author's opinion as cells tend to clump spontaneously.

Isoagglutination has also been found to occur in low salt concentrations (128, 101), too, as well as the agglutination of B. typhi by its immune serum (117). According to Aladjem and Liebermann (2) rabbit anti-chick-ovalbumin precipitates its homologous antigen in a milieu free from electrolytes.

The behaviour of plant agglutinins in hypertonic salt solutions is of some interest. Regarding isoagglutination it has been shown

onilla varia against B red cells determined by different methods.

Coronilla varia test cells suspended in										
NaCl 0.9%	AB serum 25%	50%	100%	albumin 5%	10%	20%	kollidon 2.5%	3.6%	5%	papain treated cells in saline
—	+	+++	+++	++	+++	+++	—	+	—	+
—	++	++	+++	+	+++	+++	—	+	—	+
—	++	++	+++	—	+++	+++	—	—	—	++
—	+	++	++	—	+	+++	—	—	—	++
—	+	+	++	—	—	++	—	—	—	+
—	—	+	+	—	—	+	—	—	—	+
—	—	—	+	—	—	—	—	—	—	+
—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—

earlier that a rise in salt concentration is accompanied by a fall in the agglutination titre (113, 68). This was thought to be due to an incomplete linkage of the agglutinins and damaged cells. As far as is known those studies have not included NaCl solutions stronger than 4.5 %.

The tests carried out by the present author show that when the NaCl concentration is raised from the molarity 0.15 (0.9%) the titre of most plant agglutinins falls until a concentration of 4.7 % is reached, after which the titre rises again in higher concentrations. In the higher concentrations the agglutination has a somewhat different appearance, the aggregates are smaller, but all the cells are agglutinated anyway. In isoagglutination the author has come across a similar phenomenon (101), which it is difficult to explain. The possibility of damage to the cells must be taken into account. The agglutinins, however, seem to have a share in the clumping in all salt concentrations because, in all the tests, the control tubes were negative, and because the blood group specificity of the extracts was retained completely even in high salt concentrations. It is possible, of course, that if the share of the agglutinin in the process of clumping becomes

smaller and that of the salt greater, when the salt concentration rises, the sum of their joint effects reaches its minimum in a 4.7 % solution.

As regards the effect of pH on agglutination, the present author's results speak against the theory of Kossowitch and Canat (68), according to which an agglutination would be possible only if the charges of the agglutinin and the red cell are of different designations. As the isoelectric point of many plant agglutinins is at a slightly acid pH (140, 125), their charges and those of the red cells are of the same designation, in the alkaline reaction at least. Agglutination, however, occurs in them as readily as in acid solutions.

The present author's observations are in agreement with the results from an agglutination by Phaseolus multiflorus extract obtained by Rosenthal (129). His claim that agglutination occurs at a pH as low as 3 is perhaps due to Rosenthal's measuring the pH of only the buffer used, ignoring the buffering effect of the agglutinin and maybe of the red cells, too. All cells used by the author were hemolysed at pH 3 almost instantaneously.

The behaviour of the anti-A agglutinins of Vicia cracca and Vicia villosa at various pH values is of interest. They exhibited a very definite rise in the titre upon transition from an acid to an alkaline reaction. No other plant agglutinin studied showed a similar phenomenon. Even Vicia cracca agglutinated human 0 cells and the red cells of rabbit and guinea pig as readily in acid as in alkaline solution. The anti-A agglutinin could be set free from the cells by lowering the pH of the milieu sufficiently. The agglutinin was not destroyed by the acidity because the solution, after readjustment of the pH to 7.5, agglutinated A_1 cells strongly. But it agglutinated the red cells of guinea pig, too, which goes to prove that the two agglutinins reacting differently are in the same molecule, after all.

A weak point in the present author's methods for detecting incomplete agglutinins is the fact that the cells are suspended in an albumin or kollidon solution that is less dilute than the final agglutination mixture. This may involve damage to the cells. As the purpose was to devise a method for examining about 1500 extracts, efforts were aimed at finding a technique

that would enable the author to use the saline seed extract in all the tests. This was necessary to save labour as well as seeds.

Plant agglutinins of the incomplete type seem to behave differently from animal agglutinins when determined by various methods. Animal incomplete agglutinins as a rule yield rising titres according as serum, albumin, or enzyme treatment method is used. Total failure of agglutination is common in serum milieu. In the author's studies in seed agglutinins the reaction was almost strongest in AB serum, less strong by papain and albumin methods, the kollidon method being the poorest of all.

The relationship between plant agglutinins and plant hemolysins is discussed at the end of the next chapter, taking the author's own observations into consideration.

smaller and that of the salt greater, when the salt concentration rises, the sum of their joint effects reaches its minimum in a 4.7 % solution.

As regards the effect of pH on agglutination, the present author's results speak against the theory of Kossowitch and Canat (68), according to which an agglutination would be possible only if the charges of the agglutinin and the red cell are of different designations. As the isoelectric point of many plant agglutinins is at a slightly acid pH (140, 125), their charges and those of the red cells are of the same designation, in the alkaline reaction at least. Agglutination, however, occurs in them as readily as in acid solutions.

The present author's observations are in agreement with the results from an agglutination by Phaseolus multiflorus extract obtained by Rosenthal (129). His claim that agglutination occurs at a pH as low as 3 is perhaps due to Rosenthal's measuring the pH of only the buffer used, ignoring the buffering effect of the agglutinin and maybe of the red cells, too. All cells used by the author were hemolysed at pH 3 almost instantaneously.

The behaviour of the anti-A agglutinins of Vicia cracca and Vicia villosa at various pH values is of interest. They exhibited a very definite rise in the titre upon transition from an acid to an alkaline reaction. No other plant agglutinin studied showed a similar phenomenon. Even Vicia cracca agglutinated human 0 cells and the red cells of rabbit and guinea pig as readily in acid as in alkaline solution. The anti-A agglutinin could be set free from the cells by lowering the pH of the milieu sufficiently. The agglutinin was not destroyed by the acidity because the solution, after readjustment of the pH to 7.5, agglutinated A_1 cells strongly. But it agglutinated the red cells of guinea pig, too, which goes to prove that the two agglutinins reacting differently are in the same molecule, after all.

A weak point in the present author's methods for detecting incomplete agglutinins is the fact that the cells are suspended in an albumin or kollidon solution that is less dilute than the final agglutination mixture. This may involve damage to the cells. As the purpose was to devise a method for examining about 1500 extracts, efforts were aimed at finding a technique

that would enable the author to use the saline seed extract in all the tests. This was necessary to save labour as well as seeds.

Plant agglutinins of the incomplete type seem to behave differently from animal agglutinins when determined by various methods. Animal incomplete agglutinins as a rule yield rising titres according as serum, albumin, or enzyme treatment method is used. Total failure of agglutination is common in serum milieu. In the author's studies in seed agglutinins the reaction was almost strongest in AB serum, less strong by papain and albumin methods, the kollidon method being the poorest of all.

The relationship between plant agglutinins and plant hemolysins is discussed at the end of the next chapter, taking the author's own observations into consideration.

II OCCURRENCE OF AGGLUTININS IN PLANTS

SURVEY OF LITERATURE

1. OCCURRENCE IN FLOWERING PLANTS

In seeds

Great and immediate as the interest of serologists and bacteriologists in plant agglutinins was, only few plant species containing them were found in the 19th century. After the school of Kobert (66, 67) had discovered their handful of agglutinating and also toxic species, it was not until after a decade and a half that new plant species were found containing agglutinins.

The first nontoxic and agglutinating plant extracts were discovered by Landsteiner and Raubitschek (89) in 1907, in the seeds of bean, pea, lentil, and vetch. Eisler and Portheim (42) examined the seeds of 99 species using chiefly the red cells of rabbit, sheep and goat as test cells. The species represented 56 genera of different families. They found agglutinins in but 6 datura species. Agglutinins were present in capsule-bearing daturas only, not in the bacciferous species.

All the species examined by Renkonen (122) in 1948 were of the family Leguminosae, which had turned out to be the richest source of agglutinins. Of the seed extracts 62 were inactive, 31 were unspecific agglutinants of human red cells, and 6 blood group specific. Renkonen's results are shown in detail by Table 5, which includes information of the Leguminosae species thus far examined. The 215 species, again, examined by Munter (110) belonged to 59 more families. In the families Rosaceae, Saxifragaceae, Crassulaceae, Polygonaceae, Nymphaeceae, and Lythraceae he found 20 new species that contained weak unspecific agglutinins.

Krüpe (84) examined 76 species representing 34 families, exclusive of the Leguminosae, finding no agglutinins at all. The series of Boyd and Reguera (20) comprised 262 species from 63 families. Their results, too, are indicative of the scarcity of

agglutinins outside the family Leguminosae. They found a good anti-A agglutinin in a couple of Phaseolus limensis samples, and a weak anti-B in Vitis aestivalis (Vitaceae). They noticed that different specimens of the same species may possess dissimilar activities. Kirchner and Krüpe (65, 84) confirmed this observation by examining 92 different varieties of Phaseolus limensis. They also found that of the individual beans of a variety some might contain powerful agglutinins whereas others were inactive. Differences were possible even between beans taken from one and the same pod. In 1950 Boyd (21) published a new series, which consisted of the seeds of some 300 Egyptian plant species. They did not discover new blood group specific agglutinins.

Cazal and Lalaurie (30) confined their efforts to an investigation into the family Leguminosae. They examined 420 species using human A, B and O red cells. Of the species 326 were inactive and 85 contained unspecific agglutinins. They found 9 new species containing blood group specific agglutinins. Krüpe (79) examined the seeds of 168 leguminous species using also the red cells of sheep, rabbit, guinea pig, cow, and hog, in addition to human A_1, A_2, B, and O cells. He discovered several new species exhibiting anti-H activity[1]. A survey of his results shows the almost regular occurrence of an agglutinin of the type anti-man, anti-rabbit, anti-guinea pig, anti-hog in the seeds of the tribe Vicieae (Vicia, Pisum, Lens, Lathyrus, Abrus).

In 1953 Ottensooser and Silberschmidt (114) made the striking observation that the seeds of Vicia graminea, which grows in Brazilian woods, contained an agglutinin with a specific affinity for the agglutinogen N.

In 1954 Schmidt (131) detected an agglutinin, which agglutinated strongly human B and O red cells, in the seeds of Evonymus vulgaris (Celastraceae). The agglutinin was found to be present in the aril but totally absent in the cotyledons. In 1955 Bird (15) published a compendium of the seeds of Indian plants

[1]) Animal anti-O agglutinins are commonly divided into two groups: those which are inhibited by the saliva of a secretor person, called anti-H, and those which are not, called anti-O. So far as is known to the present author, plant anti-O agglutinins discovered thus far all belong to the former group.

examined by him. It comprised over 100 species, among them Dolichos biflorus, in whose seeds he had previously found an anti-A agglutinin (5). In 1955 Ottensooser (115, 116) studied 25 Brazilian Vicia species and found an anti-A agglutinin in the seeds of Vicia peregrina. He also used the red cells of 11 animal species and noticed marked differences between the agglutination spectra of various Vicia species. Tiggelman-van Krugten et coll. (144) studied the agglutinin content of leaves principally, but also of the seeds of many species. As test cells they used the red corpuscles of chicken, guinea pig, sheep, and human A, B and O cells without finding new distinctly blood group specific seed agglutinins.

In other parts of the plant

Eisler and Portheim (43) examined the milky juice of 47 Euphorbiaceae species using the red cells of 7 animal species and found unspecific agglutinins in 26. The seeds of all species but one proved inactive. Continued studies (44) led them to the conclusion that agglutinins occur in those parts of the plant, where protein is accumulated as reserve nutrient. Agglutinins had been detected in seeds containing reserve nutrient, in potato tubers (103) and in the milky juice, which they also thought was to be regarded as reserve nutrient. The agglutinins make their appearance in seeds at a certain stage of development, they increase with an increase in protein, and vanish as the reserve nutrient is used up by the germinating seeds.

Reports on the occurrence of agglutinins in the leaves and stems of seed plants are contradictory. The preparate called robin, which was found by Lau (93) to agglutinate the red cells of different animals, was made from the bark of Robinia pseudoacacia. Kritschewsky (74) reported a discovery in the leaves of Echeveria Scheideckeri of substances agglutinating bacteria. According to Marchal (102) and Krüpe (84) this »agglutination« was due to tannin contained in the leaves. Eisler and Portheim (44) did not find agglutinins in the leaves of Phaseolus multiflorus nor, as a rule, in the stems, whereas Cazal and Lalaurie (30) reportedly detected weak agglutinins in the leaves, stems and tops of Vicia cracca. Despite his repeated

attempts Krüpe (84) has never been able to demonstrate agglutinins in the leaves, stems or roots of seed plants.

In 1956 Tiggelman-van Krugten et coll. (144) determined the agglutinin content of 313 plant extracts, the majority of which had been prepared from leaves. They reported that most of the extracts contained agglutinins. As part of the leaf extracts agglutinated the cells of different animal species very selectively, the phenomenon cannot be easily ascribed to tannin. The agglutinins, however, stood boiling for 5 to 60 minutes, so at any rate they are not substances identical with seed agglutinins.

2. OCCURRENCE IN NONFLOWERING PLANTS

In fungi

The occurrence of hemolysing agents in fungi has been known for a long time (1). The first fungous agglutinins were found by Ford (49). Galli-Valerio and Bornard (55) attempted a differentiation between fungous species on the basis of the varying agglutinative properties. Their endeavours, however, proved futile. Several mass investigations into fungous agglutinins have been carried out since then (45, 4, 84). The first blood group specific fungous agglutinins were discovered by Elo et coll. (45). Marasmius oreades was found to contain a fair anti-B agglutinin, and Hygrophorus hypothejus and Psilocybe spadicea an anti-A+B.

Ottensooser (115) did not find agglutinins in such fungi familiar to bacteriologists as Absidia, Alternaria, Aspergillus flavus, Aspergillus niger, Candida albicans, Cephalosporium, Chaetomium, Hormodendrum, Monascus purpureus, Mucor, Penicillium, Rhodotorula, Rhizopus, and Saccharomyces cerevisiae.

In lichens

Estola and Vartia (47) studied the agglutinins of one hundred Finnish lichenous species using different human erythrocytes in saline and AB serum milieu. They found unspecific agglutinins in 8 species, of which 5 belonged to the genus Peltigera.

In bacteria

Neter's survey (111) of hemagglutination caused by bacteria was published recently. Neter points out that there seems to be an almost total lack of correspondence between the taxonomic system and occurrence of agglutinins in bacteria. Many bacterial hemagglutinins exhibit definite selectivity towards the red cells of different animals.

A marked difference between bacterial and seed agglutinins consists in the former being distinctly more thermolabile. Treatment for half an hour at +56°C does not reduce the activity of seed agglutinins in the least (84), while it destroys several bacterial hemagglutinins (73, 111). Similar observations have been made concerning the agglutinins of viruses. Blood group specific bacterial hemagglutinins have not been discovered so far as the author knows.

3. BOTANICAL OBSERVATIONS

Mention was made of the studies by Eisler and Portheim above, on the basis of which they came to the conclusion that agglutinins are found in those parts of plants where proteins are accumulated as reserve nutrient.

Renkonen and Therman (124) made an interesting observation when they noticed that anti-A specific agglutinins occur in the seeds of those Vicia cracca specimens the number of whose chromosomes (2n) was 28, 14 or 27. If the number was 24 or 12, the seeds contained unspecific agglutinins. This finding was recently confirmed by Ottenscooser (115).

As yet it is not known how agglutinins are produced in a plant and what their function is, even guesses have been put forward by serologists only. Punin (118) suggests that the agglutinins are actual antibodies detailed to counteract soil bacteria. In support of his view Punin presents the fact, which he has observed, that Bacillus botulinus and B. histolyticus are agglutinated by the extract from the seeds of Laburnum alpinum. The anti-H agglutinin was absorbed by these bacteria as were also the agglutinins by O red cells against the bacteria.

Krüpe (84) calls attention to the affinity of the agglutinins for monosaccharides and oligosaccharides (which affinity will be

treated later) and suggests that botanists undertake to examine the function of the agglutinins as binders of carbohydrates.

Krüpe (75) hints at a possible relationship between the agglutinins of the leguminous species and the bacteria of the genus Rhizobium, which are found in nodules on the roots of the Leguminosae. Ottensooser (115) says that agglutinins occur chiefly in the family Leguminosae and thinks it possible that Rhizobium infection has stimulated the production of antibodies.

We have found that the extracts from Trifolium pratense, Pisum sativum, Vicia faba and Phaseolus vulgaris seeds do not agglutinate their own Rhizobium leguminosarum strains nor the strains of one another. Agglutination of human red cells by these extracts was not inhibited by bacterial suspensions nor by bacterial extracts (100).

OWN INVESTIGATIONS

1. OCCURRENCE OF AGGLUTININS IN THE SEEDS OF THE FAMILY LEGUMINOSAE

The present author studied the occurrence of hemagglutinins in the seeds of leguminous plants. He had at his disposal 1408 samples of seeds representing 743 species from 165 genera.

Material and methods

Part of the seed samples were gathered in the environs of Helsinki. The plants were identified by means of flowering specimens. Part of the samples was secured from the Botanical Garden, University of Helsinki. The greater part of the seeds, however, were received from the principals of different botanical gardens in the six continents by Professor Renkonen, M.D., Head of the Department of Serology and Bacteriology, University of Helsinki, who placed them at the author's disposal. Considering the origin of these samples it seems safe to assume that the species were correctly identified in general. By way of doublechecking, the appearance of the seeds was compared and in a few cases the seeds were sown and grown in the Botanical Garden, University of Helsinki. As the grown plants could not be brought to flower, recognition of the species was impossible, whereas that of the genera was possible with a high degree of probability. No evidence could be found pointing to wrong identification by the dispatcher. The seed samples studied were nearly all well ripened. In some cases the author had to make do with only 100 mg of seeds, which considerably inconvenienced the work.

The human red cells representing the different blood groups and used as test cells were secured among the typed personnel of the Department of

Serology and Bacteriology. The red cells of sheep, rabbit, guinea pig, and chicken were obtained from healthy, intact test animals of the Department. The bovine blood was obtained from the State Veterinary Laboratory. Samples taken from a given animal species could be from different individuals at different times.

The technique employed in the following mass investigation was chosen on the basis of the tests described in Chapter I. A method as simple as possible was strived after by the author as he was known to be in for at least 10 000 titrations.

The method chosen was practically identical with that described on page 19. The pH of the crude extracts varied from 5 to 7. It was decided, however, that unbuffered solution would be used because: 1) Agglutination by most extracts was found above to be largely independent of pH. 2) In not a single case were higher titres obtained with the buffered extracts compared with those obtained with the unbuffered extracts. 3) A fully reliable standard would not have been available by which to choose the pH of the buffer to be used.

The tests were carried out at room temperature (18°—20°C). The incubation time was 1.5—2 hours. If an extract proved inactive, the tubes were allowed to remain at +4°C for two more hours, after which they were read again. Agglutination was read with the naked eye.

Following methods were chosen for the detection of incomplete agglutinins.

1) Agglutination in AB serum milieu. This had turned out to produce results comparable to the albumin method at least, besides, it was cheaper and more convenient. Two drops of 2 % red cell suspension in AB serum was added to one drop of seed extract in saline. The final serum concentration was thus 2/3.

2) Use of papain treated red cells. The red cells were prepared by the method described in Chapter I. Two drops of 2 % suspension in saline was added to one drop of seed extract in saline.

3) Agglutination in kollidon (polyvinylpyrrolidon) milieu. The red cells were suspended in 3.6 % kollidon solution, which contained 0.9 % NaCl. Two drops of the red cell suspension in kollidon-saline was added to one drop of seed extract in saline.

Methods 2 and 3 were employed in order to find out if also among plant agglutinins there are incomplete agglutinins demonstrable by these methods but incapable of agglutinating intact cells in saline or AB serum milieu.

Five kinds of human red cells as well as the red cells of cow, sheep, rabbit, guinea pig, and chicken were used for test cells in the tests that belong to this chapter. The human cells represented the blood groups A_1, B, 00, MM, NN, S, ss, P, pp, Rh+ (D), Rh— (dd), Le(a+b—), and Le(a—b+). All the extracts were tested with the human red cells in saline and AB serum milieu. The extract was used undiluted, in saline milieu also at a dilution of 1/16, so as to allow for the possible prozone phenomenon. If agglutination or hemolysis occurred, the extract was titrated against all the cells. On the basis of the results obtained the extracts were divided into three groups:

1) Extracts that showed no visible effect on the red cells examined when these two methods were employed. If enough seeds were available, the samples were still examined by means of three kinds of human red cells representing the blood groups A_1, B, OO, MM, NN, P, pp, Le(a+b—), and Le(a—b+) using the papain and kollidon methods. They were further examined with the red cells of the above animals by the saline method. If, in these tests, any of the extracts yielded markedly different titres when various human erythrocytes were used, they were transferred to group 3.

2) Extracts that either agglutinated or hemolysed human red cells in saline or AB serum milieu but whose titres against different kinds of red cells were identical. About one third of these were tested, as in the first group, with three kinds of human red cells using both the papain and kollidon methods as well as with the above animal cells in saline. Because this group included numerous samples of closely related seeds and because, as it turned out, the papain and kollidon tests did not detect blood group specific agglutinins in this group, these additional investigations were not carried out into all seed samples. Closely related seeds in many cases also showed similar agglutination characteristics and selectivity towards the different animal cells. For this reason the samples of this group were not all tested against the animal cells either.

3) Extracts that either in saline or in AB serum milieu agglutinated human red cells selectively. If enough seeds were available, all such samples were tested by the papain and kollidon methods using different human red cells as well as by means of the above animal cells in saline. These seed samples were also subjected to further investigation in order that a more detailed study of their specificity might be possible. These tests are described in Chapter III.

Results

The results are given in Table 5. The sequence of the species in it is that of the taxonomic system. The subdivision of Willis

TABLE 5

A list of the Leguminosae seeds examined, by the present author or by other investigators, with regard to the hemagglutinin content.

Explanations:

If the results from this investigation, given by different samples of a species are reasonably similar, they are given on the same line. If the results differ from one another appreciably, they are given on two lines and the number of both types of samples is stated.

In the cases where the reasonably similar results from the author's different samples of a species are all presented on the same line, the samples are represented by a specimen giving an average agglutination titre.

Earlier data on species not examined in this study are presented on their appropriate lines each, among the other results. Their names are in parenthe-

ses immediately followed by the corresponding number of reference. If there are several results from studies by earlier authors and these conform to one another, the most extensive or earliest is presented in the table, the other reference numbers are given in the last column.

If there are earlier data on species studied in this investigation, in agreement with the present author's results, the references will be found in the last column. In the cases where earlier results do not conform to those of the author, they are presented according to the same principles as those species which were not studied in this investigation.

The order of the subfamilies and tribes follows that adopted by Willis (149). The genera of a given tribe and the species of a given genus are in alphabetical order. If a species has several names, the most commonly accepted is used as a rule. If a species is called by different names in earlier authors, these synonyms are given.

The places of origin of the author's seed samples are indicated in the third column with numbers which refer to the code below. It was not possible to ascertain the place of origin in some cases. If the samples of a species were numerous, there was not enough space in the table for every number of the place of origin.

NaCl = red cells in saline
serum = red cells in serum
papain = papain treated red cells in saline
koll. = red cells in 3.6 % kollidon-saline
— = failure of the extract to cause an agglutination or a hemolysis
+ = positive agglutination, titres not published
w = weak agglutination by undiluted extract
A, B, etc. = anti-A, anti-B, etc. specificity observed
h = hemolysis

The denominator of the final dilutions causing a clear agglutination is given in the table.

No.	*Place of origin*	*Country*	No.	*Place of origin*	*Country*
1	Achimota	Ghana	16	Bonn	Germany
2	Adelaide	Australia	17	Bratislava	Czechoslov.
3	Algiers	Algeria	18	Braunschweig	Germany
4	Alma Ata	USSR	19		Brazil
5	Amsterdam	Netherlands	20	Bremen	Germany
6	Angers	France	21	Brussels	Belgium
7	Antwerp	Belgium	22	Budapest	Hungary
8	Barcelona	Spain	23	Buenos Aires	Argentina
9	Basle	Switzerland	24	Bydgoszcz	Poland
10	Bergen	Norway	25	Canberra	Australia
11	Berkeley	U.S.A.	26	Cantonspark	Netherlands
12	Berlin	Germany	27	Chelsea	England
13	Bern	Switzerland	28	Coimbra	Portugal
14	Bialystock	Poland	29	Colonia	Uruguay
15	Bogor	Indonesia	30	Copenhagen	Denmark

No.	*Place of origin*	*Country*
31	Darmstadt	Germany
32	Debrecen	Hungary
33	Dijon	France
34	Dresden	Germany
35	Essen	,,
36	Firenze	Italy
37	Frankfurt a.M.	Germany
38	Fulda	,,
39	Gatersleben	,,
40	Giessen	,,
41	Glasgow	England
42	Göteborg	Sweden
43	Göttingen	Germany
44	Graz	Austria
45	Greifswald	Germany
46	Halle	,,
47	Hamburg	,,
48	Heidelberg	,,
49	Helsinki	Finland
50	Istanbul	Turkey
51	Kingston	Jamaica
52	Kirstenbosh	South Africa
53	Kobe	Japan
54	Köln	Germany
55	Lausanne	Switzerland
56	Leiden	Netherlands
57	Liege	Belgium
58	Lille	France
59	Lisbon	Portugal
60	Lisle	U.S.A.
61	Ljubljana	Yugoslavia
62	Lodz	Poland
63	London	England
64	Los Angeles	U.S.A.
65	Lyons	France
66	Mainz	Germany
67	Marburg	,,
68	Minden	,,
69	Montreal	Canada
70	Moorestown	U.S.A.
71	Moscow	USSR
72	Nancy	France
73	Nantes	,,
74	New York	U.S.A.
75	Northampton	U.S.A.
76	Osaka	Japan
77	Oslo	Norway
78	Ottawa	Canada
79	Pradubice	Czechoslov.
80	Paris	France
81	Peking	China
82	Potsdam	Germany
83	Poznan	Poland
84	Prague	Czechoslov.
85	Pretoria	South Africa
86	Prühonice	Czechoslov.
87	Rennes	France
88	Rome	Italy
89	Rostock	Germany
90	Saharanpur	Indian U.
91	Saitama-Ken	Japan
92	St. Gallen	Switzerland
93	Sapporo	Japan
94	Singapore	Malaya
95	Stockholm	Sweden
96	Sydney	Australia
97	Tashkent	USSR
98	Tenerife	Canary Is.
99	Tokyo	Japan
100	Toronto	Canada
101	Turku	Finland
102	Uppsala	Sweden
103	Vacratot	Hungary
104	Valentine	U.S.A.
105	Vancouver	Canada
106	Warsaw	Poland
107	Washington	U.S.A.
108	Wien	Austria
109	Wroclaw	Poland
110	Würzburg	Germany
111	Zagreb	Yugoslavia

Continued

TABLE 5 (cont'd)

A list of the Leguminosae seeds examined, by the present author or by other investigators, with regard to the hemagglutinin content
(Explanatory notes are on the first pages of the table.)

	No. of author's seed samples	Origin of the seed samples	Titres against the red cells of									Confirming results (ref. no.)
			man				cow	sheep	rabbit	guinea pig	chick	
			NaCl	serum	papain	koll.	in saline					
MIMOSOIDEAE												
Ingeae												
Albizzia amara Boiv.	1		h	—	h	h	h	h	h	h	h	
— distachia Vent.	1	108	—	—	—	—	—	—	—	—	—	
— (falcata) (122)			—									
— julibrissin Durazz.	3	81	h	—	h	h	h	h	h	h	h	30
— ,, var. speciosa Koidz.	1	53	h	—	h	h						
— Lebbeck Benth., syn. Acacia Lebbeck Willd.	3	1, 51	h	—	h	h	h	h	h	h	h	30
— lophanta Benth.	2	9, 31	—	—	—	—	—	—	—	—	—	122, 79
— (lucida) (122)			—									
— (procera Benth.) (30)			—									
— stipulata Boiv.	1		h	—	h	h	h	h	h	h	h	
— sp?	1	15	h	—	h	h						
Calliandra haematocephala Hassk.	1	15	h	—	h	h						
— Harrissii Benth.	1	19	—	—	—	—	—	—	—	—	—	
— portoricensis Benth.	1	51	—	—	16	16						
Enterolobium cyclocarpum Griseb.	1	51	h	—	h	h						
— Timboüra Mart.	1	3	h	—	h	h						
Inga vera Willd.	1		—	—	—	—	—	—	—	—	—	
(Pithecolobium albicans Benth.) (30)			—									
— arboreum Urb.	1	51	h	—	h	h						
— grandiflorum Benth.	1	96	—	—	—	—	—	—	—	—	—	
— hystrix Benth.	1	15	h	—	h	h	h	h	h	h	h	
— unguis-cati Benth	1	51	h	h	h	h	h	h	h	h	h	

Acacieae												
Acacia abyssinica Hochst.	1	64	w	—	w	w						
— (adunca A. Cunn.) (30)			—									
— aneura F. Muell.	1	25	—	—	—	—	—	—	—	—	—	
— (arabica Willd.), syn. A. Adansoni (122)			—									15
— baileyana F. Muell.	3	8, 25, 64	—	—	—	—	—	—	—	—	—	20
— brachystachya Benth.	1	96	—	—	—	—	—	—	—	—	—	
— buxifolia A. Cunn.	1	25	—	—	—	—	—	—	—	—	—	
— calamifolia Sweet.	1	25	—	—	—	—	—	—	—	—	—	
— cardiophylla A. Cunn.	1	64	—	—	—	—	—	—	—	—	—	
— (Cebil Griseb.) (30)			—									
— cultriformis A. Cunn.	1	8	—	—	—	—	—	—	—	—	—	20, 30
— (cyclops A. Cunn.) (30)			—									
— (dealbata Link.) (30)			—									
— decurrens Willd.	2	25	—	—	—	—	—	—	—	—	—	20
— Dietrichiana F. Muell.	1	36	—	—	—	—						
— discolor Willd.	2	25, 96	—	—	—	—	—	—	—	—	—	
— (dodonaeifolia Willd.) (30)			—									
— elata A. Cunn.	1	96	—	—	—	—	—	—	—	—	—	
— (falcata) (79)			—				—	—	—	—		30
— Farnesiana Willd.	3	1, 3, 15	—	—	—	—	—	—	—	—	—	30
— gladiiformis A. Cunn.	2	25	—	—	—	—	—	—	—	—	—	
— horrida Willd.	2	8, 52	—	—	—	—	—	—	—	—	—	30
— hybrida dealbata Hort.	1	8	—	—	—	—	—	—	—	—	—	30
— (hypogaea) (20)			—									
— implexa Benth.	1	25	—	—	—	—	—	—	—	—	—	
— (juniperina Willd.) (30)			—									
— laeta R. Br.	1		—	—	—	—	—	—	—	—	—	
— linearis Sims.	1	36	—	—	—	—						
— linifolia Willd.	1	96	—	—	—	—	—	—	—	—	—	
— longifolia Willd.	2	25, 36	—	—	—	—	—	—	—	—	—	20
— ,, trinervata Willd.	1	8	—	—	—	—	—	—	—	—	—	

TABLE 5 (cont'd) (Explanations are on the first pages of the table.)

	No. of author's seed samples	Origin of the seed samples	Titres against the red cells of									Confirming results (ref. no.)
			man				cow	sheep	rabbit	guinea pig	chick	
			NaCl	serum	papain	koll.	in saline					
Acacia lunata Sieber	1	25	—	—	—	—	—	—	—	—	—	30
— maidenii F. Munck.	1	96	—	—	—	—	—	—	—	—	—	
— melanoxylon R. Br.	1	8	—	—	—	—	—	—	—	—	—	30
— (moniliformis Griseb.) (30)			—									
— neriifolia A. Cunn., syn. A. retinoides	1	8	—	—	—	—	—	—	—	—	—	30
— (notabilis F. Muell.) (30)			—									
— oxycedrus Sieber	1	96	—	—	—	—	—	—	—	—	—	
— penninervis Sieber	1	25	—	—	—	—	—	—	—	—	—	
— podalyriaefolia A. Cunn.	1	8	—	—	—	—	—	—	—	—	—	30
— (pubescens R. Br.) (30)			—									
— pycnantha Benth.	1	25	—	—	—	—	—	—	—	—	—	20
— rubida A. Cunn.	1	25	—	—	—	—						
— (saligna Wendl.) (30)			—									
— (Siebertiana Hort.) (30)			—									
— (spectabilis A. Cunn.) (30)			—									
— stenophylla A. Cunn.	1	64	—	—	—	—	—	—	—	—	—	
— (verticillata Willd.) (79)			—				—	—	—	—		30
— vestita Ker.-Gawl.	1	64	—	—	—	—	—	—	—	—	—	
Eumimoseae												
Desmanthus brachylobus Benth.	1	65	—	—	—	—	—	—	—	—	—	
Leucaena glauca Benth.	3	1, 51, 85	—	—	—	—	—	—	—	—	—	30, 15
Mimosa acanthocarpa Poir.	1	8	—	—	—	—	—	—	—	—	—	
— polycarpa Kunth.	1	19	h	—	h	h						30
— pudica L.	4	2, 8, 70	—	—	—	—	—	—	—	—	—	20
— schomburgkii Benth.	1	19	h	—	h	h	h	h	h	h	h	
— (Spegazzinii Pirrota) (30)			—									
— velloziana Mart.	1	19	—	—	—	—	—	—	—	—	—	
Schrankia uncinata Willd.	1	59										

Schrankia uncinata Willd.	1	59	—	—	—	—	—	—	—	—	—	
Adenanthereae												
Adenanthera pavonina L.	1	15	—	—	—	—	—	—	—	—	—	30, 15
Dichrostachys nutans Benth.	1		—	—	—	—	—	—	—	—	—	
Neptunia oleracea Lour.	1		—	—								
Prosopis juliflora DC.	1		—	—								
Tetrapleura tetraptera	1	1	h	—	h	h	h	h	h	h	h	
Piptadenieae												
(Entada gigalobium DC.) (30)			—									
Piptadenia peregrina Benth.	1	15	h	—	h	h	h	h	h	h	h	
Parkieae												
(Parkia biglobosa Benth.) (30)			—									
— filicoidea Welw.	1	1	w	—	w	w	—	—	16	32	—	
— gigantocarpa Ducke	1	19	—	—	—	—	—	—	—	—	—	
— Javanica Mert.	1	15	—	—	—	—	—	—	16	32	—	
(Pentaclethra macrophylla Benth.) (30)			—									
II CAESALPINIOIDEAE												
Dimorphandreae												
Erythrophloeum guineense G. Don.	1	1	h	—	h	h						
Cynometreae												
(Copaifera cornui Heck.) (30)			—									
— nitida Mart.	1	19	—	—	—	—	—	—	—	—	—	
— (salicornuda) (30)			—									
Sindora scamensis Feysm.	1	15	—	—	—	—						
Amherstieae												
Afzelia africana Sm.	1	1	—	—	—	—	—	—	—	—	—	
(Saraca indica L.) (144)			128					256		256	128	

TABLE 5 (cont'd) (Explanations are on the first pages of the table.)

	No. of author's seed samples	Origin of the seed samples	Titres against the red cells of man				cow	sheep	rabbit	guinea pig	chick	Confirming results (ref. no.)
			NaCl	serum	papain	koll.	in saline					
(Schotia brachypetala Sond.) (30)			—									
— latifolia Jacq.	2	2, 3	—	—	—	—	—	—	—	—	—	
Tamarindus indica L.	1	49	—	—	—	—						122
Bauhinieae												
Bandeiraea simplicifolia Benth.	1	1	B	B	B	B	64	—	2048	h	—	99
Bauhinia aculeata L.	1	3	—	—	—	—						
— **Bonatiana**	1	81	—	N	8	4						
— **candicans** Benth.	2	8, 22	—	N	64	8	—	—	—	256	—	30
— **purpurea** L.	2	59, 90	—	N	64	4	—	—	256	—	—	99
— racemosa Lam.	1	3	w	4								
— tomentosa L.	1	1	128	128			—	—	—	—	—	122
— **variegata** L.	2	2	—	N								
— ,, var.candida	1	2	2	4			—	—	64	—	—	
— sp?	2	64, 15	—	—	—	—	—	—	512	—	—	
Cercis canadensis L.	1	79	—	—	—	—	—	—	—	—	—	20, 79
— chinensis Bunge	4	74, 81, 93	—	—	—	—	—	—	—	—	—	
— siliquastrum L.	3	7, 54, 78	—	—	—	—	—	—	—	—	—	122, 79
— (siliquastrum L.) (20)			+									
Cassieae												
Cassia absus L.	1		—	—	—	—	—	—	—	—	—	
— acutifolia Delile	1	49	—	—	—	—	—	—	—	—	—	30
— alata L.	2	1, 23	—	—	—	—	—	—	—	—	—	
— (alata L.) (144)			8					8		4	4	
— angustifolia Vahl.	1	49	—	—	—	—	—	—	—	—	—	122, 79
— appendiculata Vog.	1	19	—	—	—	—	—	—	—	—	—	
— artemisioides Gaudich.	2	64	—	—	—	—	—	—	—	—	—	20

— (atomaria L.) (30)			—									
— australis Sims.	1	96	—	—	—	—	—	—	—	—	—	
— bicapsularis L.	1		—	—	—	—						
— carnaval Speg.	1	23	—	—	—	—	—	—	—	—	—	
— chamaecrista L.	2	64, 70	—	—	—	—	—	—	—	—	—	
— (corymbosa Lam.) (30)			—									
— eremophila A. Cunn.	2	25, 96	—	—	—	—	—	—	—	—	—	
— ferruginea Schard.	1	19	—	—	—	—	—	—	—	—	—	
— fistula L.	2	1	—	—	—	—	—	—	—	—	—	20
— (grandis L.) (30)			—									
— Hookeriana Gillies	1		—	—								
— laevigata Willd.	1	99	—	—	—	—	—	—	—	—	—	
— linearifolia G. Don.	1	65	—	—	—	—	—	—	—	—	—	
— marylandica L.	2	65, 70	—	—	—	—	—	—	—	—	—	21, 30
— (Mexicana Jacq.) (30)			—									
— multijuga Rich.	1	19	—	—	—	—	—	—	—	—	—	
— nodosa Buch.-Ham.	1		—	—	—	—	—	—	—	—	—	
— nomame Honda	1	91	—	—	—	—	—	—	—	—	—	
— (obovata Coll.) (30)			—									
— occidentalis L.	2	76, 81	—	—	—	—	—	—	—	—	—	30
— rotundifolia Pers.	1	85	—	—	—	—	—	—	—	—	—	
— sieberiana DC.	1	1	—	—	—	—	—	—	—	—	—	30
— Sophera L.	3	8, 91	—	—	—	—	—	—	—	—	—	30
— (tomentosa L.) (30)			—									
— tora L.	5	1, 36, 91	—	—	—	—	—	—	—	—	—	30
Ceratonia siliqua L.	2	2, 8	—	—	—	—	—	—	—	—	—	30
Dialium guineense Willd.	1	1	—	—	—	—	—	—	—	—	—	
Eucaesalpinieae												
(Caesalpinia bonducella Flem.) (30)			—									
— (coriaria) (122)			—									
— ferrea Mart.	1	19	—	—	—	—	—	—	—	—	—	
— Gilliesii Wall.	3	23, 36, 50	—	—	4	2	—	—	—	—	—	30
— ,,	1		—	—	—	—	—	—	—	—	—	

TABLE 5 (cont'd) (Explanations are on the first pages of the table.)

	No. of author's seed samples	Origin of the seed samples	Titres against the red cells of									Confirming results (ref. no.)
			man				cow	sheep	rabbit	guinea pig	chick	
			NaCl	serum	papain	koll.	in saline					
Caesalpinia pulcherrima Sw.	4	1, 8, 64	—	—	—	—	—	—	—	—	—	122
— sepiaria Roxb.	3	51, 53, 81	—	—	—	—	—	—	—	—	—	30
— (tinctoria Domb.) (30)			—									
Delonix regia Raf., syn. Poinciana r.	2	51, 59	—	—	—	—	—	—	—	—	—	30, 15
(Gleditschia caspica) (30)			—									
— heterophylla Bunge	1	78	—	—	—	—	—	—	—	—	—	
— (inermis) (20)			—									
— japonica Miq.	1	99	—	—	—	—	—	—	—	—	—	30
— monosperma Walt.	1	74	—	—	—	—	—	—	—	—	—	
— sinensis Lam.	2	64, 78	—	—	—	—	—	—	—	—	—	
— triacanthos L.	4	60, 78, 86	—	—	—	—	—	—	—	—	—	20, 79
Gymnocladus canadensis Lam.	5	37, 60, 111	—	—	—	—	—	—	—	—	—	
— chinensis Baill.	1	81	—	—	—	—	—	—	—	—	—	
Parkinsonia aculeata L.	3	1, 8, 59	—	—	—	—	—	—	—	—	—	20
Peltophorum ferrugineum	1	1	—	—	—	—	—	—	—	—	—	
— inerme L.	1	15	—	—	—	—	—	—	—	—	—	
(Wagatea spicata) (30)			—									
III. PAPILIONATAE												
Sophoreae												
Baphia pubescens Hook.	1	1	64	2	512	128	—	—	64	512	64	
Barklya syringifolia F. Muell.	1	96	w	—	w	w	w	w	w	w	w	
Calpurnia lasiogyne E. Mey.	1	52	2	2	8	32	—	—	4	—	1	
— villosa Harv.	1	52	—	—	—	—	—	—	—	—	—	
Castanospermum australe A. Cunn.	1	98	—	—	—	—	—	—	—	—	—	
Cladrastis amurensis Benth.	1	4	2^{14}	2^{14}	2^{17}	2^{16}	128	2^{14}	2^{16}	2^{16}	2^{16}	79
— buergeri Raf.	1	99	32	2	1024	128	—	16	4	32	4	

— tinctoria Raf., syn. C. lutea K. Koch	2	60, 78	—	—	16	2	—	—	2	1	—	20, 30
Ormosia dasycarpa Jacq.	1	15	—	—	—	—	—	—	—	—	—	
Sophora chrysophylla Seem.	1		—	—								
— Davidii	1	104	—	—	—	—	—	—	—	—	—	
— flavescens Ait.	5	12, 91, 95	—	—	—	—	—	—	—	—	—	30
— **japonica** L.	5	8, 28, 106	—	A+B	A+B	A+B	—	—	256	8	—	20, 77, 79
— Moorcroftiana Benth.	3	55, 104	—	—	4	—	—	—	—	—	—	30
— secundiflora Lag.	2		—	—								
— tetraptera J. Mill.	7	43, 81	—	—	—	—	—	—	—	—	—	
— (tomentosa L.) (30)			—									
— viciifolia Hance	1	28	—	—			—	—	—	—	—	
Virgilia divaricata	1	52	—	—	H	H	8	16	—	1	8	99
— **lutea** Michx.	1	3	H	H	H	H	—	—	—	—	—	99
— **oroboides**	1	52	H	H	H	H	2	2	—	—	2	99
Podalyrieae												
(Anagyris foetida L.) (30)			—									
Baptisia australis R. Br.	6	75, 89, 105	—	—	—	—	—	—	—	—	—	122, 20, 79
— leucophaca Nutt., syn. B. bracteata Muhl.	1	60	—	—	—	—	—	—	—	—	—	
— (leucophaca) (21)			+									
— (mollis) (30)			—									
— tinctoria R. Br.	2	70	—	—	—	—	—	—	—	—	—	30
Brachysema lanceolatum Meissn.	1	2	—	—	—	—	—	—	—	—	—	30
Chorizema cordatum Lindl.	1	2	—	—	—	—	—	—	—	—	—	
— ilicifolium	1	2	—	—	—	—	—	—	—	—	—	
Cyclopia subternata Vog.	1	52	—	—	—	—	—	—	—	—	—	
— tenuifolia Lehm.	1	52	—	—	—	—	—	—	—	—	—	
Daviesia latifolia	1	2	—	—	—	—	—	—	—	—	—	
Dillwynia ericifolia Sm.	1	25	—	—	—	—	—	—	—	—	—	
Gompholobium grandiflorum Sm.	1	96	—	—	—	—	—	—	—	—	—	
— latifolium	1	2	—	—	—	—	—	—	—	—	—	
Mirbelia pungens A. Cunn.	1	25	—	—	—	—	—	—	—	—	—	
— reticulata Sm.	1	25	—	—	—	—	—	—	—	—	—	

TABLE 5 (cont'd) (Explanations are on the first pages of the table.)

	No. of author's seed samples	Origin of the seed samples	Titres against the red cells of									Confirming results (ref. no.)
			man				cow	sheep	rabbit	guinea pig	chick	
			NaCl	serum	papain	koll.	in saline					
Oxylobium callistachys Benth.	1	27	—	—								30
— ellipticum R. Br.	2	25	—	—	—	—	—	—	—	—	—	
Piptanthus nepalensis Sweet.	1	107	—	—	—	—	—	—	—	—	—	30
Podalyria calyptrata Willd.	1	52	—	—	—	—	—	—	—	—	—	
— sericea R. Br.	2	52, 70	—	—	—	—	—	—	—	—	—	
Pultenaea altissima F. Muell.	1	25	—	—	—	—	—	—	—	—	—	
— angustifolia Williamson	2	25	—	—	—	—	—	—	—	—	—	
— vrolandii Maiden	1	25	—	—	—	—	—	—	—	—	—	
Thermopsis alterniflora Rgl. et Schmalh.	1	97	—	—	—	—	—	—	—	—	—	
— (arenosa A. Nels.) (30)			—									
— caroliniana M. A. Curt.	2	70	—	—	—	—	—	—	—	—	—	30
— dolichocarpa V. Nikit.	1	97	—	—	—	—	—	—	—	—	—	
— fabacea DC.	1	37	—	—	—	—	—	—	—	—	—	30
— mollis M. A. Curt.	1	60	—	—	—	—	—	—	—	—	—	
— montana Nutt.	2	10, 92	—	—	—	—	—	—	—	—	—	30
Viminaria denudata Sm.	1	2	—	—	—	—	—	—	—	—	—	
Genisteae												
Adenocarpus intermedius DC., syn. A. complicatus Gay	3	33, 107	—	—	—	—	—	—	—	—	—	30
Argyrolobium Andrewsianum Steud.	1	2	—	—	w	—	—	—	—	—	—	
Bossiaea heterophylla Vent.	1	25	—	—	—	—	—	—	—	—	—	
— microphylla Sm.	1	25	—	—	—	—	—	—	—	—	—	
Calycotome Grosii Pau et F.-Q.	1	8	w	—	64	4	—	—	—	—	—	30
— spinosa Link.	1	80	w	—	64	8	—	—	—	—	—	30
Crotalaria compista	1	85	A+B	A+B			—	—	—	—	—	
— costata	1	85	—	—	—	—	—	—	—	—	—	
— granatiana Harv.	1	85	—	—	—	—	—	—	—	—	—	

— incana L.	1	36	—	—	—	—	—	—	—	—	—	30
— intermedia Kotschy	1	85	—	w	2	—	—	—	64	—	—	
— juncea L.	1	85	32	32	512	64	—	—	128	—	—	
— (juncea L.) (30)			—									15
— laburnifolia L.	1	85	—	—	—	—	—	—	—	2	—	
— madurensis Wight	1		—	—	—	—	—	—	256	—	—	
— mitchellii Benth.	1	96	128	64	512	2048	—	—	8192	—	—	
— paulina Schrank.	1	85	w	w	w	w	—	—	—	—	—	
— recta Steud.	1	85	2	—	16	2	—	—	16	—	—	
— retusa L.	2	1, 15	—	—	—	—	—	—	—	—	—	
— (sagittalis L.) (30)			—									
— sericea Retz.	1	85	—	—	—	—	—	—	—	—	—	
— **striata** Schrank.	2	52, 85	A+B	A+B	A+B	A+B	—	—	—	—	—	17, 144
— **usaramoensis**	2	64, 85	A+B	A+B	A+B	A+B	—	—	64	—	—	
— verrucosa L.	1	85	32	8	512	128	—	—	128	—	—	
(Cytisus albus) (122)			+									
— (**albus**) (79)			H				—	—	—	—		
— **argenteus** L.	1	36	H	H			—	—	—	—	—	30
— austriacus L., syn. C. rochelii Gris. et Schenk.	1	105	—	w			—	—	256	—	—	122
— (**austriacus**) (79)			H				—	—	—	—		
— **benehoavensis** Svent.	1	98	H	H			—	—	—	—	—	
— **biflorus** L'Herit., syn. C.elongatus Waldst. et Kit., C. ruthenicus, C. ratisbonensis Scheff.	3	21, 58, 107	H	H			—	—	4	—	—	122,79
,,	1	78	w	—	32	4	—	—	1	—	—	122
— canariensis Steud., syn. Genista canariensis L.	1	98	—	—								122
— (**canariensis**) (79)			H				—	—	—	—		
— (,, var. racemosus) (122)			—									
— candicans Lam.	4	8, 28	—	—	—	—	—	—	—	—	—	30
— (cantabricus) (30)			—									
— **capitatus** Scop., syn. C. ciliatus	2	58, 65	—	H			—	—	16	—	—	
— (capitatus) (79)			—				—	—	—	—		

TABLE 5 (cont'd) (Explanations are on the first pages of the table.)

	No. of author's seed samples	Origin of the seed samples	Titres against the red cells of									Confirming results (ref. no.)
			man				cow	sheep	rabbit	guinea pig	chick	
			NaCl	serum	papain	koll.	in saline					
Cytisus caucasicus Osb.	1	77	H	H								
— (decumbens) (79)			—				—	—	—	—		
— (emeriflorus) (30)			—									
— fragrans Lam.	1	2	—	—	—	—	—	—	—	—	—	
— (grandiflorus DC.) (30)			—									
— (hillebrandtii Briq.) (30)			—									
— (hirsutus) (79)			—				—	—	—	—		122
— linifolius Lam.	1	2	—	—	4	8	—	—	—	—	—	
— **lusitanicus** F.-Q.	1	107	H	H	64	64	—	—	32	—	—	
— (lusitanicus) (79)			—				—	—	—	—		30
— maderensis Masf.	1	28	—	—	—	—	—	—	—	—	—	30
— ,, var. magnifoliosus	1	2	—	1	32	16	—	—	2	—	—	
— monspessulanus L.	3	55, 107	—	—	—	—	—	—	—	—	—	30
— **(multiflorus)** (21)			H									
— **nigricans** L.	2	46, 78	H	H			—	—	—	—	—	79
— ,,	1	21	—	—								30
— ,, var. elongatus	1	27	H	H								
— **osmarensis** F.-Q.	1	8	H	H			—	—	—	—	—	
— (osmarensis F.-Q.) (30)			—									
— pendulinus Lf.	1	28	w	w								30
— (podolicus Blocki, var. obscura) (30)			—									
— (ponticus Griseb.) (30)			—									
— **praecox** Bean.	1	10	H	H			—	—	—	—	—	122, 79
— proliferus L.	1	98	32	32	512	64	—	—	64	—	—	
— ,, var. palmensis Christ.	1	98	4	4								
— **purpureus** Scop.	1	40	—	H			—	—	—	—	—	122, 79
— (racemosus Nichols) (30)			—									
— scoparius Link.	3	8, 58, 62	—	w	16	16	—	—	—	—	—	122, 21, 79

— scoparius Link.	2	93, 107	—	—	8	—	—	—	4	—	—	
— **sessilifolius** L.	10	44, 108, 110	H	H	H	H	—	—	—	—	—	122, 79
— (Spachianus) (30)			—									
— **supinus** L.	2	49, 78	—	H			—	—	4	—	—	122, 79
— **Weldeni** Vis.	1		—	—	H	H	—	—	—	—	—	30
— Welwitschii	1	107	—	—	—	—	—	—	—	—	—	
Genista aethnensis DC.	3	42, 88, 107	64	2	256	256	—	32	16	16	16	122
— **ancistrocarpa** Spach.	1		H	H	H	H						
— **anglica** L.	1		H	H								
— **aristata,** Presl., syn. G. dalmatica Bartl. et Wendl.	1	55	H	H								30
— (berberidea Lange) (30)			—									
— (cinerea DC.) (30)			+									
— **clavata** Poir.	1	8	H	H			—	—	—	—	—	
— (falcata Brot.) (30)			—									
— **germanica** L.	1	12	—	—	H	8	—	—	—	—	—	30
— hispanica L.	1		—	—								
— **horrida** DC.	1	108	H	H								
— (humifusa) (30)			—									
— (lusitanica L.) (30)			—									
— (mantica Poll.) (30)			—									
— **monosperma,** syn. Retama m.	5	37, 59, 64	—	H	H	H?	—	—	—	—	—	30
— ovata Waldst. et Kit.	2	55	—	—	—	—	—	—	—	—	—	
— pilosa L.	1	70	—	—	—	w						30, 79
— polygalaefolia DC.	1	104	w	w								
— radiata Scop., syn. Cytisus radiatus	2	43, 112	—	—	—	—	—	—	—	—	—	30
— (**radiata**) (79)			H				—	—	—	—		
— (rumelica) (30)			—									
— **sagittalis** L.	11	9, 37, 41	H	H	H	H	—	—	—	—	—	21, 79
— (sagittalis) (30)			—									
— (sibirica L.) (30)			—									
— **silvestris** Scop. var. dalmatica Bartl.	1		H	H								
— **sphaerocarpa** Lam.	2	8, 59	H	H								
— (sphaerocarpa) (30)			—									

TABLE 5 (cont'd) (Explanations are on the first pages of the table.)

	No. of author's seed samples	Origin of the seed samples	Titres against the red cells of									Confirming results (ref. no.)
			man				cow	sheep	rabbit	guinea pig	chick	
			NaCl	serum	papain	koll.	in saline					
(Genista tinctoria) (79)			—				—	—	—	—		122
— ,, var. hirsuta DC.	1		—	—	w	—						
— **triacanthos** Brot.	1	28	H	H								
— (triacanthos Brot.) (30)			—									
— triangularis Willd.	1	111	—	—	w	w	—	—	—	—	—	
— **umbellata** Poir.	1	8	H	H								
— virgata Link.	1	107	—	—	1	2						
Goodia lotifolia Salisb.	1	25	—	—	—	—	—	—	—	—	—	
— pubescens Sims.	1	44	—	—								
Hovea longifolia R. Br.	3	25	—	—	—	—	—	—	—	—	—	
(Laburnum adamii Kirchn.) (30)			—									
— **alpinum** J. Presl.	4	41, 77	H	H	H	H	—	—	—	—	—	122, 75, 79
— (alpinum) (20)			—									
— (Alschingeri) (122)			—									
— **anagyroides** Medic.	1	10	w	H								122, 75, 79
— (**falcoburgense**) (79)			H				—	—	—	—		
— (vulgare) (79)			H?				—	—	—	—		65, 30
— **Watereri** Dipp.	3	21, 26, 74	H	H			—	—	—	—	—	
— (Watereri Dipp.) (30)			—									
Lebeckia simsiana Eckl.	1	52	—	—	—	—	—	—	—	—	—	
Lotononis Bainesii Baker	1	85	—	—	—	—	—	—	—	—	—	
— cytisoides Benth.	1	52	32	—	32	256	—	8	256	4	16	
Lupinus affinis Agardh	1		—	—	—	—	—	—	—	—	—	30
— albus L.	1	43	—	—	—	—	—	—	—	—	—	30, 79
— angustifolius L.	1	49	—	—	2	2	—	—	16	—	—	122
— (angustifolius) (79)			—				—	—	—	—		
— (arboreus Sims.) (30)			—									
— Cruckshanksii Hook.	1		—	—	—	—	—	—	—	—	—	30

— (densiflorus Benth.) (30)			—									
— (Douglasii) (30)			—									
— (Douglasii Agardh) (30)			+									
— (elegans H. B. et K.) (30)			—									
— (hirsutus) (79)			—				—	—	—	—		
— (insignis Hort., syn. L. hybridus) (122)			—									30
— (luteus L.) (79)			—				—	—	—	—		30
— microcarpus Sims.	1	11	—	—	—	—	—	—	—	—	—	
— (mutabilis) (79)			—				—	—	—	—		30
— (nanus Dougl.) (30)			—									
— (ornatus Dougl.) (30)			—									
— perennis L.	2	70	—	—	—	—	—	—	—	—	—	30
— (pilosus Murr.) (30)			—									
— polyphyllus Link.	4	4, 10, 101	—	—	—	—	—	—	—	—	—	122, 20, 79
— (Rothmaleri Klink) (30)			—									
— Russell hybs.	1	75	—	—	—	—	—	—	—	—	—	
— subcarnosus Hook.	1	32	—	—	—	—	—	—	—	—	—	30
Petteria ramentacea Liebm.	3	9, 86, 100	—	H	H	H	—	—	—	—	—	79
— ,,	1	111	H	H	H	H	—	—	—	—	—	
Priestleya hirsuta	1	52	—	—	—	—	—	—	—	—	—	
Sarothamnus scoparius Wimm.	3	62, 88	—	—	8	2	—	—	4	—	—	79
Spartium junceum L.	4	54, 88, 107	—	—	8	2	—	—	—	—	—	20, 79
Spartocytisus filipes W. et B.	1	98	—	—	—	—	—	—	—	—	—	
— nubigenus W. et B.	1	98	—	—	—	—	—	—	—	—	—	
Templetonia retusa R. Br.	1	2	—	—	—	—						30
Ulex densus Welw.	1	28	H	H								
— **europaeus** L.	4	43, 70, 117	H	H	H	H	—	—	—	—	—	30, 79
— **Gallii** Planch.	1	28	H	H								
— **Jussiaei** Webb.	1	55	H	H								30
— (micranthus Lange) (30)			—									
— **nanus** Forst.	1	28	H	H								
— (**provincialis** Loisel) (30)			H									

TABLE 5 (cont'd) (Explanations are on the first pages of the table.)

	No. of author's seed samples	Origin of the seed samples	Titres against the red cells of									Confirming results (ref. no.)
			man				cow	sheep	rabbit	guinea pig	chick	
			NaCl	serum	papain	koll.	in saline					
Trifolieae												
(Medicago arabica L.) (30)			—									
— arborea L.	1	80	h	—	h	h						
— (cardiensis) (79)			—				—	—	—	—		
— carstiensis Wulf.	1	108	—	—	—	—	—	—	—	—	—	30
— (ciliaris Krocker) (79)			h				h	h	h	h		30
— coerulea Less.	1	71	—	—	—	—	—	—	—	—	—	
— (echinata Spandl.) (30)			—									
— (echinus L.) (30)			—									
— (gerardi Willd.) (30)			—									
— (helix Willd.) (30)			—									
— hispida Gaertn., syn. M. polycarpa	2	29, 39	—	—	—	—	—	—	—	—	—	30
— intertexta Mill.	1		—	—	—	—	—	—	—	—	—	
— ,,	1	49	h	—	h	h	h	h	h	h	h	122
— laciniata Mill.	1		—	—	—	—	—	—	—	—	—	30
— (lappacea Desr.) (30)			—									
— (littoralis Rhode) (30)			—									
— lupulina L.	1	101	h	—	h	h	h	h	h	h	h	30, 79
— (maculata Willd.) (30)			—									
— (marina L.) (30)			—									
— minima L.	4	17, 32, 54	—	—	—	—	—	—	—	—	—	30
— (obscura Retz. ssp. helix Urb. var. inermis Urb.) (30)			—									
— (officinalis) (122)			—									
— orbicularis All.	1	54	—	—	—	—	—	—	—	—	—	30, 79
— rigidula Desr.	3	39, 97	h	—	h	h	h	h	h	h	h	
— (rugosa Desr.) (30)			—									
— sativa L., syn. M. media Pers.	4	29, 49, 103	—	—	—	—	—	—	—	—	—	122, 20, 30, 79

— sativa	1	97	h	—	h	h	h	h	h	h	h	
— scutellata Mill.	1	49	—	—	h	h	—	h	h	h	h	122
— truncatula Gaertn., syn. M. Murex Willd.	1	85	—	—	h	h	—	—	—	—	—	30, 79
— (tuberculata) (79)			h				h	h	h	h		
— turbinata Willd.	1		h	—	h	h	h	h	h	h	h	30
Melilotus alba Desr.	3	29, 49, 78	—	—	—	—	—	—	—	—	—	122, 20, 79
— indica All., syn. M. parviflora Desf., Trigonella melilotus Link.	1	39	—	—	—	—	—	—	—	—	—	122, 30
— italica Lam.	1	102	—	—	—	—	—	—	—	—	—	30
— officinalis Lam.	4	4, 101, 107	—	—	—	—	—	—	—	—	—	122, 79
— (sicula Jacks.) (30)			—									
— sulcata Desf.	2	39, 102	h	—	h	h	h	h	h	h	h	
— ruthenica Ser.	2	32	—	—	—	—	—	—	—	—	—	
Ononis alopecuroides L.	1	65	—	—	—	—	—	—	—	—	—	
— (arborescens) (30)			—									
— (arvensis L.) (30)			—									
— (biflora Desf.) (30)			—									
— fruticosa L.	1	70	—	—	—	—	—	—	—	—	—	30, 79
— hebecarpa W. et B.	1	98	—	—	—	—	—	—	—	—	—	
— hircina Jacq.	1		—	—								30
— natrix L.	2	2, 57	—	—	—	—						30, 79
— (porrigens Salzm.) (30)			—									
— (repens L.) (79)			—				—	—	—	—		30
— rotundifolia L.	2	39	—	—	—	—	—	—	—	—	—	30
— (spinosa L.) (30)			—									
— ulicina W. et B.	1	98	—	—	—	—						
Parochetus communis Buch.-Ham.	1	70	—	—	—	—	—	—	—	—	—	
(Trifolium agrarium L.) (79)			—				—	—	—	—		30
— alexandrinum L.	1	29	—	—	—	—	—	—	—	—	—	30
— (ambiguum M. B.) (30)			—									
— (angustifolium L.) (30)			—									
— (badium Schreber) (30)			—									
— (Bocconii Savi) (30)			—									

TABLE 5 (cont'd) (Explanations are on the first pages of the table.)

	No. of author's seed samples	Origin of the seed samples	Titres against the red cells of									Confirming results (ref. no.)
			man				cow	sheep	rabbit	guinea pig	chick	
			NaCl	serum	papain	koll.	in saline					
(Trifolium cernuum Brot.) (30)			—									
— (diffusum Ehrh.) (30)			—									
— dubium Sibth., syn. T.minus Rethan.	1	29	—	—	—	—	—	—	—	—	—	30
— (filiforme L.) (30)			—									
— fragiferum L.	2	29, 85	—	—	—	—	—	—	—	—	—	
— (glomeratum L.) (30)			—									
— (Heldreichianum Hausskn.) (30)			—									
— hirtum All.	1	85	—	—	—	—	—	—	—	—	—	
— hybridum L.	1	49	—	—	—	—	—	—	—	—	—	122
— incarnatum L.	3	29, 49	—	—	—	—	—	—	—	—	—	122, 79
— (isthmocarpum Brot.) (30)			—									
— (lappaceum L.) (30)			—									
— (ligusticum Balb.) (30)			—									
— (maritimum Huds.) (30)			—									
— medium L.	1	49	—	—	—	—	—	—	—	—	—	30,79
— (Michelianum Savi) (30)			—									
— (Miegeanum Maire) (30)			—									
— (montanum L.) (79)			—				—	—	—	—		30
— (nigrescens Viv.) (30)			—									
— (ochroleucum Huds.) (30)			—									
— (pannonicum) (79)			—				—	—	—	—		
— (panormitanum Presl.) (30)			—									
— (parviflorum Ehrh.) (30)			—									
— (patens Schreber) (30)			—									
— (physodes Stev.) (30)			—									
— polymorphum Poir.	1	29	—	—	—	—	—	—	—	—	—	
— pratense L.	3	29, 49	—	—	—	—	—	—	—	—	—	122, 79
— procumbens L.	1	29	—	—	—	—	—	—	—	—	—	30

— repens L.	3	29, 49	—	—	—	—	—	—	—	—	—	122, 20, 79
— (resupinatum L.) (30)			—									
— (rubens L.) (79)			—				—	—	—	—		30
— rueppellianum Fresen.	1	85	—	—	—	—	—	—	—	—	—	
— (scabrum L.) (30)			—									
— semipilosum Fresen.	1	85	—	—	—	—	—	—	—	—	—	
— (stellatum L.) (30)			—									
— (strictum W. et Kit.) (30)			—									
— subterraneum L.	2	29, 85	—	—	—	—	—	—	—	—	—	30
— (suffocatum L.) (30)			—									
— (tomentosum L.) (30)			—									
(Trigonella Besseriana Ser.) (30)			—									
— calliceras Fish.	1	39	—	—	—	—	—	—	—	—	—	30
— caerulea Ser.	1	109	—	—	—	—	—	—	—	—	—	30, 79
— (caerulea) (122)			h									
— cretica Boiss.	3	49, 66	—	—	—	—	—	—	—	—	—	122
— foenum-graecum L.	4	45, 49, 107	—	—	—	—	—	—	—	—	—	122, 20, 79,15
— polycerata L.	1	102	—	—	—	—	—	—	—	—	—	
— radiata L.	1	102	—	—	—	—	—	—	—	—	—	
Loteae												
Anthyllis Barba-Jovis L.	1	2	—	—	—	—	—	—	—	—	w	30
— (Gerardi L.) (30)			—									
— Hermanniae L.	1	8	—	—	—	—	—	—	—	—	—	30
— illyrica Beck.	1	111	—	—	—	—						
— Jacquini Kern.	1	111	—	—	—	—						30
— (lotoides L.) (30)			—									
— montana L.	1	37	—	—	—	—	—	—	—	—	—	30, 79
— tetraphylla L.	2	8, 61	—	—	—	—	—	—	—	—	—	30
— Vulneraria L.	5	61, 71, 92	—	—	—	—	—	—	—	—	—	122, 79
— (,, var.alp.) (79)			—									
Dorycnium germanicum Rouy	2	108, 111	—	—	—	—	—	—	—	—	—	
— herbaceum L.	2	37, 108	—	—	—	—						30, 79

TABLE 5 (cont'd) (Explanations are on the first pages of the table.)

	No. of author's seed samples	Origin of the seed samples	Titres against the red cells of									Confirming results (ref. no.)
			man				cow	sheep	rabbit	guinea pig	chick	
			NaCl	serum	papain	koll.	in saline					
Dorycnium hirsutum Ser.	1	55	—	—	—	—	—	—	—	—	—	30
— (latifolium Willd.) (30)			—									
— rectum Ser.	1		—	—	—	—	—	—	—	—	—	30
— ,,	2	3	—	—	2	8	—	—	—	—	—	
Hosackia americana Pieper	1	68	—	—	—	—	—	—	—	—	—	
Hymenocarpos circinnata Savi	1	68	—	—	—	—						
— nummularius Willd.	1	68	—	—	—	—	—	—	—	—	—	
Lotus arabicus L. var. trigonelloides W. et B.	1	98	—	—	—	—	—	—	—	—	—	
— arenarius Brot.	1		—	w								
— **biflorus** Desr.	1	9	H	H								
— campylocladus W. et B.	1	28	—	—	—	—	—	—	—	—	—	
— conimbricensis Brot.	2	28, 102	—	—								
— corniculatus L.	6	10, 43, 69	—	—	—	—	—	—	—	—	—	
— **(corniculatus)** (79)			H				—	—	—	—		
— creticus L.	2	28	—	—	—	—	—	—	—	—	—	
— cytisoides L.	1	28	—	—								
— (edulis L.) (30)			—									
— hispidus Desf.	1	28	—	—	—	—	—	—	—	—	—	30
— Jacobaeus L.	1	80	—	—	—	—	—	—	—	—	—	
— lancerottensis W. et B.	1	98	—	—			—	—	—	—	—	
— ornithopodioides L.	4	28, 37, 56	—	—	—	—	—	—	—	—	—	30, 79
— parviflorus Desf.	1	28	—	—	—	—	—	—	—	—	—	30
— **Requieni** Fisch. et Mey.	1	72	H	H								
— ,,	1		—	—								30
— **siliquosus** L., syn. Tetragonolobus maritimus Roth.	3	16, 37, 44	H	H			—	—	—	—	—	
— ,,	2	92	—	H			—	—	2	—	—	30

— **tetragonolobus** L., syn. L. or Tetragonolobus purpureus Moench.	29	20, 24, 67	H	H	H	H	—	—	—	—	—	122, 75, 30,79
— uliginosus Schkuhr.	6	28, 47, 73	—	—	—	—	—	—	—	—	—	30, 79
— villosus Burm.	1	85	—	—	—	—	—	—	—	—	—	
— (Weilleri Maire) (30)			—									
Securigera coronilla DC., syn. S. securidaca L.	4	6, 36, 66	—	—	—	—	—	—	—	—	—	30, 79
Galegeae												
Amorpha californica Nutt.	3	12, 78, 108	—	—	—	—	—	—	—	—	—	30
— canescens Nutt.	4	4, 60, 78	—	—	—	—	—	—	—	—	—	30, 79
— fruticosa L., syn. A. nana Nutt.	9	4, 12, 86	—	—	—	—	—	—	—	—	—	30, 79
— (fruticosa) (30)			+									20
— Lewisii Lodd.	2	78, 83	—	—	—	—	—	—	—	—	—	
— microphylla Pursh.	1	83	—	—	—	—	—	—	—	—	—	
— virgata Small	1	78	—	—	—	—	—	—	—	—	—	
Astragalus adsurgens Pall.	1	69	—	—	—	—	—	—	—	—	—	30
— akkensis Coss.	1	8	—	—	—	—	—	—	—	—	—	
— alopecuroides Pall.	2	66, 71	—	—	—	—	—	—	—	—	—	30
— alpinus L., syn. A. penduliflorus	1	87	—	—	—	—	—	—	—	—	—	122
— americanus M. E. Jones	1	23	—	—	—	—	—	—	—	—	—	
— aristatus L'Herit., syn. A. sempervirens Lam.	1		—	—	—	—	—	—	—	—	—	30
— asper Jacq.	1	32	—	—	—							
— baeticus L.	2	8, 61	—	w	w	w	—	—	—	—	—	30
— (brachycarpus) (79)			—				—	—	—	—		
— canadensis L.	1	23	—	—	—	—	—	—	—	—	—	
— chinensis L.	1	65	—	—	—	—	—	—	—	—	—	
— cicer L.	5	4, 14, 62	—	—	—	—	—	—	—	—	—	122, 79
— (chlorostachys) (30)			+									
— danicus Retz.	1	61	—	—	—	—	—	—	—	—	—	30
— (depressus L.) (30)			—									
— eremospartoides Regel	1	97	—	—	—	—	—	—	—	—	—	
— excapus L.	1		—	—	—	—	—	—	—	—	—	

TABLE 5 (cont'd) (Explanations are on the first pages of the table.)

	No. of author's seed samples	Origin of the seed samples	Titres against the red cells of									Confirming results (ref. no.)
			man				cow	sheep	rabbit	guinea pig	chick	
			NaCl	serum	papain	koll.	in saline					
Astragalus eximius Bunge	1	97	—	—	—	—	—	—	—	—	—	
— falcatus Lam.	2	4, 101	—	—	—	—	—	—	—	—	—	30, 79
— (frigidus) (79)			—				—	—	—	—		
— glycyphyllos L., syn. A. galegiformis L.	4	4, 8, 66	—	—	—	—	—	—	—	—	—	30, 79
— (granatensis Lange) (30)			—									
— (gummifer Labill.) (30)			—									
— hamosus L.	2	8, 61	—	w	4	2	—	—	—	—	—	30
— hypoglottis L., syn. A. purpureus Lam.	1	68	—	—	—	—	—	—	—	—	—	30
— lusitanicus Lam.	1	8	—	—	—	—	—	—	—	—	—	30
— (massiliensis Lam.) (30)			—									
— (maximus) (79)			—				—	—	—	—		
— monspessulanus L.	2	66	—	—	w	w	—	—	—	—	—	30
— monspessulanus var. chlorocyaneus Boiss.	1	8	—	—	—	—	—	—	—	—	—	
— (odoratus Lam.) (30)			—									
— onobrychis L.	2	108	—	—	—	—	—	—	—	—	—	30
— oxyglottis Stev.	1	8	—	—	—	—	—	—	—	—	—	
— (pannosus Fenzl.) (30)			—									
— pectinatus Dougl.	1	23	—	—	—	—	—	—	—	—	—	
— pentaglottis L.	1	65	—	—	—	—	—	—	—	—	—	30
— Reinii Ball.	1	8	—	—	—	—	—	—	—	—	—	
— (Robinsii A. Gray) (30)			—									
— Sieversianus Pall.	1	97	—	—	—	—	—	—	—	—	—	
— sikkimensis Benth.	1	8	—	—	—	—	—	—	—	—	—	
— sinicus L.	1	76	—	—	—	—	—	—	—	—	—	
— sulcatus L.	3	4, 65	—	—	—	—	—	—	—	—	—	30

— (tragacanthus) (79)			—				—	—	—	—		
— (verus Oliv.) (30)			—									
— (vicioides) (30)			—									
Biserrula Pelecinus L.	2	34, 68	—	—	w	w	—	—	—	—	—	30
(Calophaca wolgarica Fisch.) (30)			—									
Caragana ambigua Stocks.	1	89	16	16								
— arborescens Lam.	2	78	32	4	128	64	—	—	64	2	2	30, 79
— (arborescens) (20)			—									
— ,, var. pendula Lam.	2	49, 78	4	4	32	8	—	4	16	—	16	
— (aurantiaca Koehne) (30)			—									
— Boisii Schneid.	1	78	16	8								30
— (Boisii) (79)			—				—	—	4	2		
— brevispina Benth.	1	89	16	16			—	8	16	—	—	30, 79
— Chamlagu Lam.	1	4	—	—	16	4	—	4	—	4	2	
— (conferta Benth.) (30)			+									
— decorticans Hemsl.	1	78	32	16	256	128	1	4	32	1	32	30, 79
— (densa Kom.) (30)			—									
— (franchettiana) (79)			64									
— frutescens Medic.	1	65	32	8								
— frutex K. Koch	2	78	32	8	256	128	—	64	—	—	16	79
— **frutex var. latifolia** Schneid.	1	78	—	A+B	A+B	A+B	—	256	—	2	—	
— maximowicziana Komar.	1	78	32	8	512	16						
— (maximowicziana Komar.) (30)			—									
— microphylla Lam.	2	33, 65	8	4	32	16	—	—	8	4	8	
— (microphylla Lam.) (79)			—				—	2	4	2		30
— pygmaea DC.	1	89	—	1	4	2						
— sibirica Medic.	1	34	32	16	128	64						30
— sophoraefolia Tausch.	2	65, 78	32	8			—	—	16	—	16	
— spinosa DC.	1	71	—	—	4	1						30
— (tragacanthoides) (30)			—									
Carmichaelia australis R. Br.	2	16, 44	—	—	—	—	—	—	—	—	—	
— Enysii T. Kirk.	2	44, 107	—	—	—	—	—	—	—	—	—	30
— flagelliformis Col.	1	44	—	—	—							30
— Munroi Hook.	1	81	—	—	—	—	—	—	—	—	—	

TABLE 5 (cont'd) (Explanations are on the first pages of the table.)

	No. of author's seed samples	Origin of the seed samples	Titres against the red cells of man				cow	sheep	rabbit	guinea pig	chick	Confirming results (ref. no.)
			NaCl	serum	papain	koll.	in saline					
Carmichaelia odorata Col.	1	45	—	—	—	—	—	—	—	—	—	30
— stricta Lehm.	1	44	—	—			—	—	—	—	—	
— subulata	2	81	—	—	—	—	—	—	—	—	—	
— Williamsi T. Kirk.	1	2	—	—	—	—	—	—	—	—	—	
Chordospartium Stevensoni	2	81	—	—	—	—	—	—	—	—	—	
Clianthus formosus	2	2, 70	—	—	—	—	—	—	—	—	—	
— puniceus Banks et Soland.	4	2, 81	—	—	—	—	—	—	—	—	—	
— ,, var. rosea	1	81	—	—	—	—	—	—	—	—	—	
— speciosus Aschers et Graebn.	1	25	—	—	—	—	—	—	—	—	—	
Colutea arborescens L., syn. C. melanocalyx Boiss.	3	74, 86, 92	—	—	—	—	—	—	—	—	—	30, 79
— arborescens L.	1	4	—	—	16	16	—	—	—	—	—	
— (brevialata Lange) (30)			—									
— cilicica Boiss.	1	9	—	—	2	—	—	—	—	—	—	30, 79
— cruenta Ait.	1	45	—	—	—	—	—	—	—	—	—	
— gracilis Freyn. et Sint.	1	54	—	—	w	—	—	—	—	—	—	79
— halepica Lam., syn. C. istria Mill.	2	34, 110	—	—	—	—	—	—	—	—	—	30, 79
— longialata	1	110	—	—	—	—	—	—	—	—	—	79
— media Willd.	2	78, 86	—	—	—	—	—	—	—	—	—	30
— orientalis Mill.	2	58, 75	—	—	—	—	—	—	—	—	—	30, 79
— persica Boiss.	3	78, 83, 110	—	—	—	—	—	—	—	—	—	30
— (violascens) (30)			—									
Corallospartium crassicaule Armst.	1		—	—	—	—	—	—	—	—	—	
(Cyamopsis psoralioides DC.) (30)			—									15
Dalea alopecuroides Willd.	2	58, 65	—	—	—	—	—	—	—	—	—	30
(Galega bicolor Hausskn.) (30)			+									
— officinalis L., syn G. persica Pers.	2	12, 70	8	—	64	128	—	—	128	64	—	122, 30, 79
— orientalis Lam.	2	101	32	1	256	512	—	—	256	128	—	30

— orientalis Lam.	2	101	32	1	256	512	—	—	256	128	—	30	
— patula Stev.	1		16	—	64	64						30	
Glycyrrhiza asperrima L.	1	97	—	—	—	—	w	—	w	w	w		
— echinata L.	2	13, 37	—	—	—	—	—	—	—	—	—	30, 79	
— (,, var. freatiris Boiss.) (30)			—										
— foetida	1	27	—	—	—	—							
— (foetidissima Tausch.) (30)			—										
— glabra L., syn. G. glanduliflera Waldst. et Kit.	4	58, 69, 97	—	—	—	—	w	—	w	w	4	122, 30, 79	
— lepidota Pursh.	1	100	—	—	—	—	—	—	—	—	—	30	
— triphylla Fisch. et Mey.	1	97	—	—	—	—	—	—	—	—	—		
— uralensis Fisch.	2	71, 97	—	—	—	—	—	—	—	—	—	30	
Halimodendron argenteum Fisch.	2	46	64	—	1024	512	—	—	512	256	—		
— halodendron Voss.	2	17, 97	16	4	128	64	—	16	64	1	16		
Indigofera amblyantha Craib.	1	74	—	—	—	—	—	—	—	—	—	30	
— (argentea Buk.) (30)			—										
— arrecta Hochst.	1	85	—	—	—	—	—	—	—	—	—		
— australis Willd.	2	25	—	—	—	—	—	—	—	—	—		
— (canescens Mort.) (30)			—										
— cylindrica DC.	1	52	—	—	—	—	—	—	—	—	—		
— cytisoides L.	1		—	—	—	—	—	—	—	—	—		
— (decora Lindl.) (30)			—										
— (Dosua Buch.-Ham.) (30)			—										
— frutescens L.	1	52	—	—	—	—	—	—	—	—	—		
— gerardiana R. Grah.	5	50, 54, 107	—	—	—	—	—	—	—	—	—	30, 79	
— (hebepetala) (30)			—										
— hirsuta Harv.	3	4, 85	—	—	—	—	—	—	—	—	—		
— (Kirilowii Maxim.) (30)			—										
— (macrostachya Vilm. et Boiss.) (30)			—										
— neglecta	1	85	—	—	—	—	—	—	—	—	—		
— (Potaninii Craib.) (30)			—										
— pseudo-tinctoria Matsumura	2	53, 99	—	—	—	—	—	—	—	—	—		
— (pulchella) (30)			—										
— retroflexa Baill.	1	85	—	—	—	—	—	—	—	—	—		
— splendens Ficalho et Hiern.	1	65	—	—	—	—	—	—	—	—	—	30	

TABLE 5 (cont'd) (Explanations are on the first pages of the table.)

	No. of author's seed samples	Origin of the seed samples	Titres against the red cells of									Confirming results (ref. no.)
			man				cow	sheep	rabbit	guinea pig	chick	
			NaCl	serum	papain	koll.	in saline					
Indigofera subulata Vahl.	1	85	—	—	—	—	—	—	—	—	—	
— (subulata) (144)			256					256		256	256	
— tinctoria	1	85	—	—	—	—	—	—	—	—	—	21
—, mixed	1	70	—	—	—	—	—	—	—	—	—	
Millettia japonica Gray	2	53, 99	16	8	1024	512	—	—	512	32	—	
— thonningii Baker	1	1	—	—	—	—	—	—	—	—	—	
Mundulea suberosa Benth.	2	15, 52	—	—	—	—	—	—	—	—	—	
(Oxytropis argentata) (79)			—				—	—	—	—		
— (argentea Pers.) (30)			—									
— campestris DC.	5	8, 42, 92	—	—	—	—	—	—	—	—	—	122, 79
— deflexa DC.	1	69	—	—	—	—	—	—	—	—	—	
— gracilis K. Schum.	2	23, 69	—	—	—	—	—	—	—	—	—	
— japonica	1	42	—	—	—							
— (Lambertii Pursh.) (30)			—									
— macounii Rydb.	1	23	—	—	—	—	—	—	—	—	—	
— megalantha	2	42, 78	—	—	—	—	—	—	—	—	—	
— monticola A. Gray	1	78	—	—	—	—	—	—	—	—	—	
— (pilosa DC.) (30)			—									
— (strobilacea Bunge) (30)			—									
— (sulphurea) (79)			—				—	—	—	—		122
— uralensis DC., syn. O. Halleri Bunge	1	102	—	—	—	—	—	—	—	—	—	30
(Psoralea americana L. var. polystachya Flori et Paoletti) (30)			—									
— bituminosa L.	5	9, 45	—	—	—	—	—	—	32	128	—	30
— ,, var. communis W. et B.	1	98	—	—	—	—	—	—	—	—	—	
— (evrylifolia) (30)			—									
— macrostachya DC.	1		—	—	—	—	—	—	—	—	—	
— pinnata L.	1	52	—	—	—	—	—	—	—	—	—	
	1	70, 74	16	8	128	256	—	64	4	16	128	30

Robinia hispida L.	2	70, 74	16	8	128	256	—	64	4	16	128	30
— holdtii Boiss.	1	78	64	16	512	512	—	128	16	16	128	
— Kelseyi	1	74	32	16								
— (luxurians Schn.) (30)			+									
— neomexicana A. Gray	1	22	16	16			—	64	8	64	64	
— pseud-acacia L.	6	60, 78, 100	32	8	64	128	—	64	8	64	32	20, 30, 79
— (pseud-acacia L. var. monophylla Carr.) (30)			—									
— pseud-acacia L. forma decaisniana Carr. Voss.	2	78, 107	16	4								
— (tortuosa DC.) (30)			+									
— viscosa Vent.	2	60, 74	64	64			—	—	1	2	—	30
Sesbania aculeata Poir.	1	85	h	—	h	h	h	h	h	h	h	
— (aegyptica) (30)			—									
— grandiflora Poir.	2	1, 15	h	—	h	h	h	h	h	h	h	
— marginata Benth.	1	23	h	—	h	h	h	h	h	h	h	
— punicea Benth.	1	70	h	—	h	h	h	h	h	h	h	
— tripetii	1	64	h	—	h	h	h	h	h	h	h	
Sutherlandia frutescens R. Br.	3	52, 70	—	—	—	—	—	—	—	—	—	30
Swainsona coronillaefolia Salisb.	3	3, 25, 59	—	—	—	—	—	—	—	—	—	30
— (galegifolia R. Br. var. violacea Hort.) (30)			—									
— greyana Lindl.	2	2, 25	—	—	—	—	—	—	—	—	—	
— (salsula Taub.) (30)			—									
Tephrosia candida DC.	1	12	—	—	w	—	—	—	—	—	—	
— elegans Schum.	1	1	—	—	—	—	—	—	—	64	—	
— glomeruliflora Meissn.	1	52	—	—	—	—	—	—	—	256	—	
— grandiflora Pers.	1	52	—	—	—	—	—	—	—	128	—	
— nyikensis Baker	1	23	—	—	—	—	—	—	—	—	—	
— Vogelii Hook.	1	15	—	—	—	—	—	—	—	—	—	
Wistaria brachybotrys Sieb. et Zucc.	1	53	16	8			—	—	16	1	2	
— chinensis DC., syn. W. sinensis Sweet.	2	64, 81	16	16	256	64	—	—	256	4	2	20
— ,, var. alba Rehd. et Wils.	1		8	4	256	16	—	—	32	—	4	
— ,, var. rosea	1	99	16	8			—	—	512	8	8	

TABLE 5 (cont'd) (Explanations are on the first pages of the table.)

	No. of author's seed samples	Origin of the seed samples	Titres against the red cells of									Confirming results (ref. no.)
			man				cow	sheep	rabbit	guinea pig	chick	
			NaCl	serum	papain	koll.	in saline					
Hedysareae												
Adesmia bicolor DC.	1	29	—	—	w	—	—	—	—	—	—	
Aeschynomene indica L.	1	16	—	—	—	—	—	—	—	—	—	30
— (sensitiva) (144)			—					—		4	4	
Alhagi sparsifolia Shop.	1	97	—	—	—	—	w	w	w	w	—	
Alysicarpus vaginalis DC.	1	3	—	—	—	—	—	—	—	—	—	
Amicia zygomeris DC.	1	98	—	—	—	—	—	—	—	—	—	30
Arachis hypogaea L.	1	37	—	—	—	—	—	—	—	—	—	15
Coronilla cappadocica Willd.	1	67	—	—			—	—	—	—	—	30
— coronata L.	3	16, 31, 66	—	—	—	—	—	—	—	—	—	
— cretica L.	2	66	—	—	—	—	—	—	—	—	—	
— (elegans Panc.) (79)			—				—	—	—	—		30
— emeroides Boiss. et Spr.	1	111	—	—	—	—	—	—	—	—	—	
— emerus L.	4	36, 44, 66	—	—	—	—	—	—	—	—	—	
— glauca L.	1		—	—	—	—	—	—	—	—	—	21, 30
— ,, var. pygmaea	1	107	—	—	—	—	—	—	—	—	—	
— (juncea L.) (30)			—									
— (minima L.) (79)			—				—	—	—	—		30
— (montana) (79)			—				—	—	—	—		
— repanda Boiss.	1	14	—	—	—	—	—	—	—	—	—	30
— scorpioides Koch	1	12	—	—	—	—	—	—	—	—	—	122, 79
— vaginalis Lam.	1		—	—	—	—	—	—	—	—	—	30
— Valentina L.	1	88	—	—	—	—	—	—	—	—	—	30
— **varia** L.	7	35, 37, 43	—	A+B	A+B	A+B	—	—	16	—	—	122, 77, 79
— (viminalis) (30)			—									
(Desmodium adscendens DC.) (30)			—									
— canadense DC.	3	4, 69	—	—	—	—	—	—	—	—	—	30, 79
— canadensis Kze	2	8	—	—	—	—	—	—	—	—	—	

— (cuspidatum Hook.) (79)			—				—	—	—	—		30
— Dillenii Darl.	1	32	—	—	—	—	—	—	—	—	—	30
— discolor Vog.	1	85	—	—	—	—	—	—	—	—	—	
— fallax	1	99	—	—	—	—	—	—	—	—	—	
— (frutescens) (144)			8					8		8	—	
— (gangeticum DC.) (30)			—									
— glutinosum Wood	1	100	—	—	—	—	—	—	—	—	—	30
— gyrans DC.	1	70	—	—	—	—	—	—	—	—	—	
— limense Hook.	1	85	—	—	—	—	—	—	—	—	—	
— (nudiflorum DC.) (30)			—									
— racemosum DC.	1	99	—	—	—	—	—	—	—	—	—	
— (sessilifolium Torr. et Gray) (30)			—									
— tiliaefolium G. Don.	1	98	—	—	—	—	—	—	—	—	—	30
— uncinatum DC.	1	98	—	—	—	—	—	—	—	—	—	30
Eversmannia Lehmanni Bunge	1	97	—	—	—	—	—	—	—	—	—	
Hedysarum alpinum L.	1	49	—	—	—	—	—	—	—	—	—	122
— alpinum var. americanum Michx.	2	49, 69	—	—	—	—	—	—	—	—	—	
— ,, var. philoscia Rollins	1	69	—	—	—	—	—	—	—	—	—	
— (boreale L. var. americanum Michx. (30)			—									
— (capitatum) (30)			—									
— coronarium L.	2	12, 16	—	—	—	—	—	—	—	—	—	30
— (esculentum L.) (30)			—									
— (flexuosum L.) (79)			—				—	—	—	—		30
— hedysaroides Sch. et Th.	1	49	—	—	—	—	—	—	—	—	—	79
— (japonicum Basin.) (30)			—									
— multijugum Maxim.	1	9	—	—	—	—	—	—	—	—	—	30
— obscurum L.	2	54	—	—	—	—	—	—	—	—	—	30
— (,, var. japonicum) (30)			—									
— Semenovii Rgl. et Herd.	1	71	—	—	—	—	—	—	—	—	—	30
— sibiricum Poir.	2	20, 68	—	—	—	—	—	—	—	—	—	122
— sulfurescens	1	105	—	—	—	—	—	—	—	—	—	
Hippocrepis ciliata Willd.	1	68	—	—	—	—	—	—	—	—	—	30
— comosa L.	1	37	—	—	—	—	—	—	—	—	—	30, 79

TABLE 5 (cont'd) (Explanations are on the first pages of the table.)

	No. of author's seed samples	Origin of the seed samples	Titres against the red cells of									Confirming results (ref. no.)
			man				cow	sheep	rabbit	guinea pig	chick	
			NaCl	serum	papain	koll.	in saline					
Hippocrepis multisiliquosa L.	1	68	—	—	—	—	—	—	—	—	—	
— (unisilicosa L.) (30)			—									
Lespedeza bicolor Turcz.	4	26, 55, 78	—	—	—	—	—	—	—	—	—	30
— Buergeri Miq.	1	53	—	—	—	—	—	—	—	—	—	
— capitata Michx.	1	100	—	—	—	—	—	—	—	—	—	
— cyrtobotrya Miq.	2	53, 99	—	—	—	—	—	—	—	—	—	30
— danvica schimadae	1	85	—	—	—	—	—	—	—	—	—	
— formosa	1	81	—	—	—	—	—	—	—	—	—	
— hirta Ell.	2	74, 100	—	—	—	—	—	—	—	—	—	
— homoloba	1	99	—	—	—	—	—	—	—	—	—	
— juncea Pers.	1	99	—	—	—	—	—	—	—	—	—	
— (macrocarpa, syn. Campylotropis macrocarpa Rehd.) (30)			—									
— nikkoensis	1	99	—	—	—	—	—	—	—	—	—	
— retusa Nakai	1	53	—	—	—	—	—	—	—	—	—	
— sericea	1	85	—	—	—	—	—	—	—	—	—	
— stipulacea	1	29	—	—	—	—	—	—	—	—	—	
— Thunbergii Nakai	1	70	—	—	—	—	—	—	—	—	—	30
— sp?	1	85	—	—	—	—	—	—	—	—	—	
Lourea vespertilionis Desv.	3	31, 43, 44	—	—	—	—	—	—	—	—	—	
Onobrychis caput-galli Lam.	1	36	—	—	—	—	—	—	—	—	—	122
— cherassanica Bunge	1	97	—	—	—	—	—	—	—	—	—	
— (crista-galli) (122)			—									
— cyri Grossh.	1	71	—	—								
— grandis Lipsky	1	97	—	—	—	—	—	—	—	—	—	
— micrantha Schrenk.	1	97	—	—	—	—	—	—	—	—	—	
— (oxytropoides Bunge) (30)			—									
— pulchella Schrenk.	1	97	—	—	—	—	—	—	—	—	—	

— saxatilis Lam.	1	24	—	—	—	—	—	—	—	—	—	
— sibirica Turcz.	1	71	—	—	—	—	—	—	—	—	—	
— transcaucasica Grossh.	1	46	—	—	—	—	—	—	—	—	—	
— viciaefolia Scop., syn. O. sativa Lam.	7	69, 71, 106	—	—	—	—	—	—	—	—	—	122, 30, 79
Ornithopus compressus L.	1	102	—	—	—	—	—	—	—	—	—	
— ebracteatus Brot.	1	65	—	—	—	—	—	—	—	—	—	
— isthmocarpus Coss.	1	68	—	—	—	—	—	—	—	—	—	
— perpusillus L.	2	65, 68	—	—	—	—	—	—	—	—	—	30
— pinnatus Mill.	2	102	—	—	—	—	—	—	—	—	—	
— sativus Brot.	1	85	—	—	—	—	—	—	—	—	—	30, 79
Scorpiurus muricata L.	1	2	—	—	—	—	—	—	—	—	—	30
— muricata var. subvillosa Thell.	1	95	—	—	—	—	—	—	—	—	—	
— subvillosa L.	1	2	—	—	—	—	—	—	—	—	—	122
— (sulcata L.) (30)			—									
— vermiculata L.	2	49, 58	—	—	—	—	—	—	—	—	—	122
Stylosanthes gracilis H. B. et K.	1	85	—	—	—	—	—	—	16	4	—	
Dalbergieae												
(Andira anthelminthica) (30)			—									
— inermis H. B. et K.	1	15	—	—	—	—	—	—	—	2048	—	
Dipteryx odorata Willd.	1	49	—	—	—	—	—	—	—	—	—	122
Lonchocarpus discolor Hub.	1	19	—	—	w	w	—	—	64	256	—	
— (neuroscapha Benth.) (30)			—									
Piscidia piscipula Sarg.	1	51	—	—	—	—	—	—	—	—	—	
(Pongamia glabra) (30)			—									15
Pterocarpus violaceus Vog.	1	19	—	—	—	—	—	—	—	—	—	
Tipuana speciosa Benth.	2	8, 59	—	—	—	—	—	—	—	—	—	
— tipu O. K.	2	23, 64	—	—	—	—	—	—	—	—	—	
Vicieae												
Abrus precatorius L.	2	1, 15	1024	32	2^{16}	2^{16}	4	—	2048	1024	512	122, 20, 15
— pulchellus Wall.	1	27	—	—	—	—	—	—	—	—	—	30
Cicer arietinum L.	4	12, 54, 108	—	—	—	—	—	—	—	—	—	122, 79, 15

TABLE 5 (cont'd) (Explanations are on the first pages of the table.)

	No. of author's seed samples	Origin of the seed samples	Titres against the red cells of									Confirming results (ref. no.)
			man				cow	sheep	rabbit	guinea pig	chick	
			NaCl	serum	papain	koll.	in saline					
(Cicer arietinum) (20)			A?									
— pinnatifidum Jaub. et Spach.	4	12, 39	—	—	w	—	—	—	—	—	—	
— (songaricum Steph.) (30)			—									
Lathyrus alatus Ten.	1	24	32	—								
— alpestris Kit.	1	9	4	—	128	64						30, 79
— angulatus L.	1	24	16	2			—	—	32	16	—	
— annuus L.	1	95	8	—	32	8						
— aphaca L.	2	10, 24	8	—								30, 79
— articulatus L.	1	24	64	—	1024	1024						30
— atropurpureus Lap.	1	87	16	—	128	128						
— cicera L.	3	12, 24, 37	16	2	128	128						30
— (cingitans) (79)			32				1	—	256	256		
— cirrhosus Ser.	1	26	16	2			—	—	64	64	—	
— (cirrhosus Ser.) (30)			—									
— clymenum L.	3	12, 18, 27	16	—	128	64	—	—	32	32	—	122, 79
— cyaneus C. Koch	1	65	8	—								30
— (filiformis) (30)			—									
— filiformis J. Gay var. pallescens M.B.	1	95	—	—	—	2	—	—	—	—	—	
— (galegiformis Hort.) (30)			+									
— Gorgoni Parl.	1		16	1	256	256						30
— (grandiflorus) (79)			8					—	64	32		30
— heterophyllus L.	1	33	4	—	8	8						30
— hirsutus L.	1	95	4	—	32	16						
— lathyroides L.	1	57	4	—	32	16						
— latifolius L.	3	7, 70, 92	8	—	64	32	—	—	64	64	—	122, 20, 79
— luteus Peterm.	2	108	8	—	64	32	—	—	128	64	—	30
— ,, ssp. transsilvaticus Fisch.	1	95	16	2								
— Magellanicus Lam.	1	26	16	—	64	32						30

— maritimus Bigel, syn. L. japonicus Willd.	4	57, 69, 99	8	—	128	64	—	—	128	128	—	122, 30
— (maritimus var. aleuticus Fern.) (30)			+									
— ,, var. glaber Fern.	1	99	32	1	64	128						
— montanus Bernh.	2	7, 16	4	—	64	32						
— neurolobus Boiss. et Heldr.	1		2	—	8	8						
— niger Bernh., syn. Orobus n. L.	5	7, 37, 108	4	—	16	8	—	—	64	64	—	122, 30, 79
— ,, var. roseus	1		8	1								
— nissolia L.	2	5, 7	16	—	64	64	—	—	256	256	—	30, 79
— ochroleucus Hook.	1	69	8	—	128	64						
— ochrus DC.	2	5, 12	16	—	256	128						30, 79
— odoratus L.	2	7	8	—	64	64	—	—	128	128	—	20, 21, 79
— palustris L.	1		4	—								30
— (palustris L.) (30)			—									
— pisiformis L.	1	95	16	—	256	64						30
— pratensis L.	3	7, 71	4	—	64	32	—	—	32	32	—	122, 79
— **pratensis** L.	1		4	A			—	—	64	32	—	
— (rotundifolius) (122)			+									
— sativus L.	2	82, 107	16	—	128	64	—	—	64	64	—	30, 79, 15
— setifolius L.	1	106	32	—	64	128						
— (sylvestris L.) (30)	3	92	8	—	128	32						122, 79
— (**sylvestris** L.) (30)			A									
— ,, var. platyphyllus Retz.	2	26, 95	4	—								
— tingitanus L.	4	18, 27, 34	16	1	64	64						122
— tuberosus L., syn. Orobus t. L.	1	7, 97	4	—	16	16						30
— (undulatus) (30)			—									
— (variegatus) (30)			+									
— varius C. Koch	2	13	16	—	32	64						
— (vehetus) (79)			2				—	1	32	32		
— venetus Wohlf., syn. Orobus v.	1	55	4	—	64	32						30
— (venetus Wohlf.) (30)			—									
— venosus Muhl. var. intonsus Butters et St. John	1	69	16	1	64	32						30
— vernus Bernh., syn. Orobus v.	5	10, 69, 105	8	—	64	64	—	—	64	64	—	122, 30, 79

TABLE 5 (cont'd) (Explanations are on the first pages of the table.)

	No. of author's seed samples	Origin of the seed samples	Titres against the red cells of man				cow	sheep	rabbit	guinea pig	chick	Confirming results (ref. no.)
			NaCl	serum	papain	koll.	in saline					
Lathyrus vernus var. gracilis	1	69	16	—	64	64						
Lens culinaris Medic., syn. Lens esculenta Moench. and Ervum Lens L.	6	14, 92, 101	64	4	512	256	—	—	256	256	—	20, 30, 79 etc.
— culinaris ssp. abyssinica Alef.	1		8	—	32	16	—	—	128	32	—	
— **nigricans** Godr.	2	102, 110	8	N			—	—	64	64	—	79
— peruviana L.	2		4	—	64	32						
(Pisum hirsutum) (79)			16				8	8	64	64		
— Jomardi Schrank.	4	2, 27	16	—	256	128	—	—	128	64	—	30
— sativum L., syn. P. arvense	9	30, 32, 39	8	—	64	64	—	4	128	16	—	122,20,30 etc.
— (Thebaicum Willd.) (30)			+									
Vicia americana Muhl.	3	23, 69, 78	1	—	16	16	—	—	256	16	—	30
— angustifolia L.	1	12	2	—								30, 79
— articulata Hornem.	1	39	32	—	64	64						
— atropurpurea Desf., syn. V. benghalensis L.	3	5, 29	16	4	512	256	—	—	512	256	—	122, 116 etc.
— aurantiaca Boiss.	1		8	—	128	128	—	—	32	32	—	
— bithynica L.	2	39, 68	16	—	64	64						30
— calcarata Desf.	4	24, 40, 72	8	—	128	64						30
— cassubica L.	3	40, 95	8	—	256	128						122, 79
— (cirrhosa) (30)			+									
— cornigera Chaub.	2	82	8	2	64	64						30
— **cracca** L.	21	21, 49	A	A			—	—	1024	512	—	122,65,30 etc.
— ,,	6	38, 63	4	—	64	64	—	—	256	128	—	124, 115
— (,, var. gerardi) (75)			—									
— dasycarpa Ten.	8	29, 79, 102	8	2	128	32	—	2	64	32	—	30
— disperma DC.	4	29, 79	8	2	64	32						30, 116
— (disperma DC.) (30)			—									
— **dumetorum** L.	3	37, 57	8	A	256	32	—	—	64	32	—	65, 30
— eriocarpa	1		—	—	8	2						

— eriocarpa	1		—	—	8	2						
— erviformis Boiss.	2	59, 103	8	—								
— ervilia Willd., syn. Ervum e.	7	39, 59, 102	16	—	128	128	—	—	128	128	—	30, 79, 116
— faba L.	3	10, 69, 103	4	—	64	64						20, 30, 79, 15
— (faba L.) (30)			—									
— ferruginea Boiss.	1		8	—	64	32						30
— (fulgens Batt.) (30)			+									
— (galeata) (116)			+					—	+		—	
— Gerardi Vill.	1	27	—	—								
— gigantea Bunge	2	100, 107	4	—	64	16						
— (gracilis Loisel) (30)			—									
— **graminea** Sm.	1	19	N	16								114, 116 etc.
— grandiflora Scop.	2	44, 102	4	—	32	16	—	—	64	32	—	30
— (grandiflora Scop.) (30)			—									
— (hircomica) (30)			+									
— hirsuta S. F. Gray	2	49	w	—	4	8	—	—	64	64	—	30
— hybrida L.	1	72	32	4	1024	1024						30
— lathyroides L.	2	73	2	—	64	64						30
— leavenworthii Torr.	1	23	4	—	64	32						
— leganyana Rap. et Leng.	2	12, 89	4	—	32	32						
— **leganyana**	2	39	16	N	32	32	—	—	64	32	—	
— (linearifolia) (114)			—									
— ludoviciana Nutt.	1		8	—	256	256						30
— (ludoviciana Nutt.) (30)			—									
— lutea L.	1	12	16	—								30, 79, 116
— ,,	1	73	—	—	—	—						
— (,, var. laevigata Boiss.) (30)			—									
— (,, ssp. muricata Ser.) (30)			+									
— macrocarpa Bertol.	1	23	4	—	16	8						
— melanops Sibth. et Sm.	2	36	32	4	128	128						65, 30
— Michauxii Spreng.	1		4	—	64	32						30
— monanthos Desf.	1	68	16	4	64	128						116
— (muricata Ser.) (30)			+									
— nana Vog.	1	23	4	—	8	16	—	—	32	32	—	

TABLE 5 (cont'd) (Explanations are on the first pages of the table.)

	No. of author's seed samples	Origin of the seed samples	Titres against the red cells of man				cow	sheep	rabbit	guinea pig	chick	Confirming results (ref. no.)
			NaCl	serum	papain	koll.	in saline					
Vicia narbonensis L.	2	12, 82	—	—	—	—	—	—	—	—	—	30, 79
— ,,	1		1	—	8	4						
— obovata Gaud.	1	68	16	1	128	64						
— onobrychioides L.	1		2	—	16	4						30
— oroboides Wulf.	2	41, 111	8	—	512	128						122
— orobus DC.	2	10, 95	4	—	16	32	—	—	16	8	—	
— (orobus) (79)			—				—	—	8	8		
— pallida Turcz.	1	99	8	—	16	16						
— pannonica Cr., syn. V. purpurascens DC., V. striata Bieb.	1	67	32	2	256	256						122, 30, 116
— **peregrina** L.	6	37, 59, 79	4	A	32	8	—	—	16	4	—	
— ,,	1	73	8	—	32	16	—	—	32	16	—	30
— (peregrina L.) (116)			A									
— picta Fisch. et Mey.	1	83	4	—	8	4						30
— **picta**	1	82	16	N	128	128						
— pisiformis L.	2	78, 102	4	—	16	16						30, 79
— pyrenaica Pourr.	1	80	2	—	32	32						
— (satica L. var. maculata Burnat) (30)			—									
— sativa L., syn. V. amphicarpa Doth., V. cordata Wulf.	30	5, 47, 95	8	2	64	16	—	—	128	128	—	122,30,79 etc.
— (sativa) (116)			—				—	—	+		—	21
— sepium L., syn. V. rotundifolia Gilib.	2	10, 97	64	—	1024	1024						122
— (seriocarpa) (114)			+				—	—	+		+	
— silvatica L.	2		2	—								122, 30
— tenuifolia Rth.	2	22, 49	8	2	1024	512	—	—	128	128	—	79
— **tenuifolia**	2	22, 84	A?	A			—	—	256	64	—	30
— ,, var. stenophylla	1	63	8	—	512	512						30
— tetrasperma L.	1	83	8	—	16	16						122, 79
— tricolor Hort.	1	72	8	2								

— tricolor Hort.	1	72	8	2								
— (tricolor Hort.) (30)			—									
— unijuga A. Br.	4	5, 93, 102	2	—	128	32	—	—	512	256	—	30, 79
— **varia** Hort.	2	24	A?	A			—	—	256	128	—	
— **villosa** Roth.	7	16, 24, 29	A	A			—	2	128	64	—	65, 79
— (villosa) (122)			+									20, 30
— ,, ssp. eu-villosa Cav.	2	95	8	—	64	32						
— (,, ssp. eu-villosa Cav.) (30)			—									
Phaseoleae												
Amphicarpaea bracteata Fern.	1	100	A	A			—	—	—	—	—	99
— **Edgeworthii** Benth., syn. A. trisperma Baker	1	99	A	A	A	A	—	—	—	—	—	99
Apios tuberosa Moench.	1	40	—	—	w		—	—	—	—	—	
Atylosia barbata Baker	5	34, 56, 62	256	256	8192	512	—	16	128	1024	4	
— ,,	1	32	—	—	—	—	—	—	—	—	—	
Cajanus cajan Millsp.	1	64	—	—	—	—	—	—	—	—	—	
— indicus Spreng.	2	85, 98	—	—	—	—	—	—	—	—	—	30, 15
Canavalia ensiformis DC.	2	30, 94	—	—	256	4	—	—	128	256	—	20, 144
— ,,	1	85	4096	4096	2^{16}	2^{16}	—	256	512	2048	64	15
Centrosema plumieri Benth.	1	3	—	—			—	—	—	—	—	122
— pubescens Benth.	1	15	—	—	—	—	—	—	—	—	—	122, 15
— virginianum Benth.	1	70	—	—	—	—	—	—	—	—	—	
Clitoria racemosa G. Don.	1	19	—	—	—	—	—	—	—	—	—	
— ternatea L.	5	15, 49, 98	h	—	h	h	h	h	h	h	h	30
Dioclea glycinoides Hort.	1	36	8	4	256	128	—	2048	1024	2048	16	
— malacocarpa Ducke	1	19	—	—	—	—	—	—	—	—	—	
Dolichos bicontortus Dur.	1	30	—	—	—	—	—	—	—	—	—	
— (bicontortus Dur.) (30)			+									
— **biflorus** L.	2	85	A	A	A	A	—	—	—	—	—	5, 30, 84
— falcatus Klein.	1	85	h	—	h	h	h	h	h	h	h	
— gibbosus Thunb.	1	52	—	—	—	—	—	—	—	—	—	
— **lab-lab** L., syn. D. lignosus L.	3	12, 85	64	A+B			—	32	512	512	32	
— ,, and D. purpureus L.	2	56, 62	512	128	2^{14}	2^{16}	—	128	1024	2048	128	20, 15

TABLE 5 (cont'd) (Explanations are on the first pages of the table.)

	No. of author's seed samples	Origin of the seed samples	Titres against the red cells of									Confirming results (ref. no.)
			man				cow	sheep	rabbit	guinea pig	chick	
			NaCl	serum	papain	koll.	in saline					
(Dolichos lab-lab L.) (30)			—									
— leucomelas Kze	1	30	—	—	—	—	—	—	—	—	—	
— lubia Forsk.	1	103	256	128								
— ,,	1	103	—	—	—	—	—	—	—	—	—	
— sesquipedalis L.	1	32	—	—	—	—	—	—	—	—	—	15
— tortuosum	1	85	—	—	—	—	—	—	—	—	—	
— (zebra Mart.) (30)			—									
(Eriosema grandiflorum G. Don.) (30)			—									
Erythrina bogotensis Hort.	1	98	32	64	256	128	—	—	—	—	8	
— crista-galli L.	5	2, 70, 98	32	16	512	128	—	—	8	—	16	21
— glauca Willd.	1	19	16	4			—	—	1	—	8	
— indica Lam.	2	94	64	64	2^{15}	2^{14}	—	—	64	—	128	30, 15
— vespertilio Benth.	1	2	32	32	4096	1024	—	—	—	—	32	
— ,,	1	64	—	—	—	—	—	—	—	—	—	
Flemingia strobilacea Ait.	2	30, 66	—	—			—	—	—	—	—	
Glycine javanica L.	1	85	—	—	—	—	—	—	—	—	—	
— soja Sieb. et Zucc., syn. G. max	7	12, 44, 81	—	2	1024	16	—	—	256	—	—	122,20,79 etc.
— ussuriensis Rgl. et Maack.	1	54	—	—	256							
Hardenbergia comptoniana Benth.	3	2, 64	—	—	—	—	—	—	—	—	—	
— monophylla Benth.	1	25	h	—	h	h	—	—	h	h	—	
— ,,	1	2	—	—	—	—	—	—	—	—	—	
— ,, var. alba	1	2	—	—	2	—	—	—	—	—	—	
—, mixed	1	70	—	—	—	—	—	—	—	—	—	
Kennedya prostrata R. Br.	1	2	—	—	—	—	—	—	—	—	—	
— rubicunda Vent.	2	2, 25	—	—	—	—	—	—	—	—	—	30
Mucuna deeringiana	3	3, 85, 94	—	—	—	—	—	—	—	—	—	
— (nivea) (15)			—									
— pruriens DC.	1	1	—	—	—	—	—	—	—	—	—	30
— (sloanei) (144)			32					64		32	8	
— (urens DC.) (30)			A?									

— (sloanei) (144)			32					64		32	8	
— (urens DC.) (30)			A?									
— utilis L.	1	44	—	—	—	—						
Pachyrrhizus angulatus Rich.	1		—	—	—	—	—	—	—	—	—	122
— bulbosus Kurz.	1	15	—	—	—	—	—	—	—	—	—	
— erosus	1	94	—	—	—	—	—	—	—	—	—	
— tuberosus Spreng., syn. Dolichos tuberosus Lam.	1	3	—	—	—	—	—	—	—	—	—	
— (tuberosus Spreng.) (30)			+									
(Phaseolus acutifolius A. Gray var. latifolius) (30)			+									
— angularis Willd.	1	23	—	—	—	—	—	1	1	—	2	
— (calcaratus) (15)			—									
— cerasiferus L.	2		512	64								79
— coccineus L.	1	32	32	32	2^{14}	4096	—	32	64	512	8	20
— ellipticus Schard.	1		256	64								
— (hysterinus) (122)			+									
— **lunatus** L., syn. P. limensis Macfad.	1	15	A	A	A	A	—	—	—	—	—	20, 21, 65, 84
— ,,	1	85	—	—	—	—	—	—	—	—	—	20
— (lunatus L.) (30)			+									20, 65, 84
— multiflorus Willd.	1		128	16								30, 79
— mungo L., syn. P. radiatus L. and	2	32, 67	256	128	2^{14}	2^{14}	—	64	256	1024	4	122, 30, 79
— ,, P. aureus Roxb.	4	23, 69, 85	—	—	—	—	—	—	—	—	—	20, 15
— Ricciardianus Ten.	1		256	64	4096	512	—	512	128	1024	4	
— (trilobus) (15)			—									
— (tuberosus) (79)			256					256	512	512		
— vulgaris L., syn. P. nanus L.	4	49	256	128			—	128	512	512	16	133, 122 etc.
Physostigma venenosum Balf.	1		—	—	—	—	—	—	—	—	—	122, 20
(Psophocarpus tetragonolobus) (122)			+									15
Pueraria hirsuta Kurz.	1	51	—	—	—	—	—	—	—	—	—	
— phaseoloides Benth., syn. P. javanica Benth.	1		—	—	—	—	—	—	—	—	—	122
— Thunbergiana Benth.	2	53, 85	—	—	—	—	—	—	—	—	—	20
Rhynchosia caribaea DC.	2	98	—	—	—	—	—	—	—	—	—	

TABLE 5 (cont'd) (Explanations are on the first pages of the table.)

	No. of author's seed samples	Origin of the seed samples	Titres against the red cells of									Confirming results (ref. no.)
			man				cow	sheep	rabbit	guinea pig	chick	
			NaCl	serum	papain	koll.	in saline					
Rhynchosia phaseoloides DC.	5	22, 48, 107	—	—	—	—	—	—	—	—	—	79
— pyramidalis	2	16, 26	—	—	—	—	—	—	—	—	—	
— tomentosa Hook. et Arn.	1	98	—	—	—	—	—	—	—	—	—	
— volubilis Lour.	1	99	—	—	—	—	—	—	—	—	—	
Teramnus labialis Spreng.	1	85	—	—	—	—	—	—	1024	2048	—	
Vigna capensis Walp., syn. Phaseolus caffer Hab.	3	2, 110	64	64	2^{14}	2^{16}	—	16	128	1024	128	30
— capensis	2	32, 85	—	—	—	—	—	—	—	—	—	
— catjang Walp., syn. V. sinensis Endl.	3	76, 85	—	—	—	—	—	—	—	—	—	30, 15, 144
— ,,	3	43, 62	512	256	2^{14}	2^{15}	—	512	512	512	32	
— **catjang** Walp.	1	32	64	A+B			—	w	128	128	8	
— cylindrica	1	26	128	128	2^{14}	4096	—	4	64	256	16	
— helvola	1	100	—	—	—	—	—	—	—	—	—	
— luteola Benth.	1	103	64	64			—	—	1024	1024	128	
— retusa Walp.	1	36	128	128	2^{15}	2048	—	32	64	64	16	
— sesquipedalis	2	2, 85	—	—	—	—	—	—	—	—	—	30
Voandzeia subterranea Thou.	1	85	—	—	—	—	—	—	—	—	—	

(149) was observed in the compilation. In addition to the author's own results, other investigators' findings on the Leguminosae were included.

Agglutinating seed samples were found in 37% of the 743 species studied by the author. When comparing the different methods it is noticed that the greater part of the seed agglutinins were demonstrable by intact red cells suspended in saline milieu (95 %). Agglutinins that eluded the saline method were largely demonstrable by intact cells suspended in serum (3 %). Still there were agglutinins that could be found only by the papain and/or kollidon methods used, in which instances the titre was low as a rule. Also because saline agglutinins were demonstrable in the concentrated agglutinin solutions in a couple of these cases (101), it would seem that the occurrence of cryptagglutinoids in seeds is infrequent, indeed. In some cases, when the titre in saline was much less than the other titres, it showed a prozone phenomenon, which was otherwise of most rare occurrence.

Determined by the papain and kollidon methods the agglutinin titres were generally higher than they were in saline and serum milieu. No great differences were usual between the papain and kollidon titres. An exception to the rule was the genus Glycine, whose agglutinins reacted decidedly better when tested by the papain method.

Titres in the serum milieu exhibited interesting diversity. They were frequently almost as high as in saline milieu. The serum also showed a definite inhibiting effect on the agglutination in many cases; this is particularly true of the agglutinins of the tribe Vicieae. The fact that the serum does not apparently inhibit blood group specific agglutinins is of practical interest.

In some cases agglutination was found to become more distinct at +4°C than it was at room temperature, but cold agglutinins, pure and simple, were not found in the Glycine species, either.

DISCUSSION

Of the sources of error in this chapter probably the most obvious is faulty identification of species by the dispatching garden. The measures that were taken in order to eliminate this source of error were described in the foregoing. Special attention was paid to cases that gave unexpected results. It is the author's belief that the probability of errors which might arise as a consequence of faulty identification has been reduced to a reasonable minimum by means of the precautions.

The diversity of botanical nomenclature is a considerable nuisance to a reader of the literature on plant agglutinins. Numerous species have been given different names by different investigators, indeed, different specimens of one and the same species have been termed by different synonyms in a single work. The matter is made worse by the fact that the appropriate author's name has not been appended to the names of the species, as a rule. Unfortunately, a total elimination of this disadvantage did not succeed in this paper, either.

Another source of error consists in the fact that in the study of how the different extracts react with the erythrocytes of different animals, the red cells of only one individual were used; even the only individual of a given animal species was not the same every day. Supposing that the red cells of different individuals of an animal species were agglutinated selectively by a plant extract, the case would not be fully illustrated by the present author's results.

The agglutination titre of a seed extract of course depends on the degree of fineness achieved when the seeds are reduced to powder. No doubt the degree varies from time to time. On the basis of his experiments the author found this factor to be of no great importance.

Still another source of error was revealed by the observation that newly harvested seeds of Bandeiraea simplicifolia yielded an extract different from that yielded by seeds of the same sample one year later (99). The author has witnessed no other phenomenon of this kind, after all. The majority of the received seeds seemed well ripened.

Quantitative variations in the agglutinins of different samples of a species seem to be often greater than qualitative. For this reason absolute titres are often of less value than relative, i.e. the titre against a kind of cells compared with that of another. When the test cells are of several kinds, the agglutination patterns of closely related plant species obtained by titration are often very much alike.

If agglutinins are scarce in the seeds of a genus or a species generally, they may be in some samples so scant that the agglutinins cannot be demonstrated by the usual means. In such cases they may be demonstrable by more sensitive methods, e.g. by papainised cells or in a concentrated extract (101).

Concerning the relationship between seed agglutinins and hemolysins Ehrlich (40) believed that the agglutinins and hemolysins of ricin and abrin were identical, and that the phenomena differ from each other only quantitatively. The present author has also noticed the tendency of red cells in highly concentrated agglutinin solutions to become partly or totally hemolysed before long (101).

There are also plant extracts that hemolyse red cells without an agglutination taking place. As shown in Table 5, extracts of this kind were obtained especially from many seeds belonging to the tribes Ingeae and Trifolieae. Apart from their immediate effect these plant hemolysins differ from plant agglutinins in other respects, too.

1) There are only few totally unspecific plant agglutinins, i.e. such as agglutinate the red cells of different animal species equally well. The titres of many plant hemolysins studied, on the contrary, against the red cells of different animal species are practically identical and low as a rule (101).

2) Most plant agglutinins are neutralised by some of the simple sugars or blood group substances. The hemolysins, contrariwise, seem to remain unaffected as will be shown in the next chapter.

For the above reasons this investigation is not going to enter deeply into the subject of plant hemolysins.

On the basis of the results given in Table 5 it seems probable that the subfamily Mimosoideae is poor in agglutinins. The

seeds of only a couple of Parkia species have been found to contain agglutinins of the type anti-rabbit+guinea pig. In the seeds of Calliandra portoricensis weak agglutinins have been detected reactive with human erythrocytes.

Agglutinins occur in one tribe, Bauhinieae, of the subfamily Caesalpinioideae. Very weak agglutinins were found by the present author in the seeds of Caesalpinia Gilliesii, too. Tiggelman -van Krugten et coll. (144) have found agglutinins also in the seeds of Saraca indica and Cassia alata, but it must be remembered that their technique differs a little from the most generally used, and that they have found agglutinins in almost all of the leguminous seeds examined by them.

The tribe Bauhinieae seems to be quite interesting. Probably the purest known anti-B plant agglutinin, which seems to improve in specificity as the age of the seeds increases, has been found in the seeds of Bandeiraea simplicifolia (99). Several Bauhinia species seem to contain anti-rabbit agglutinins. Showing some anti-N specificity many of them react more weakly with human red cells.

In the subfamily Papilionatae there are 10 tribes according to Willis's dictionary. Two of them, Podalyrieae and Trifolieae, have not been found to contain unmistakable agglutinins. In the rest of the tribes agglutinins occur more or less regularly.

The number of the examined species of the tribe Sophoreae is rather small. This tribe attracts attention by virtue of the regular occurrence, in many of the studied seed samples of Sophora japonica, of an agglutinin of the type anti-A+B+ rabbit. Another agglutinin of the type anti-H+cow+sheep+ chicken occurs in the three examined species of the genus Virgilia.

The agglutinin content of numerous seed samples of the tribe Genisteae has been determined, and one cannot help being struck by the very frequent occurrence of anti-H agglutinins in the seeds of the genera Cytisus, Genista, Laburnum, Petteria, and Ulex. As a rule these extracts have not agglutinated the red cells of the studied animal species. A few of these agglutinins, however, react with the red cells of rabbit, too. The tribe in question also includes species that agglutinate human red cells unspecifically, and their reaction with the red cells of rabbit

is stronger. Also many seeds of the genus Crotalaria contain anti-man+rabbit agglutinins. In some cases these agglutinins exhibit distinct anti-A+B specificity.

In the genus Lotus (Tetragonolobus) of the tribe Loteae there are also such agglutinins as react with those human red cells almost solely that belong to the blood groups O and A_2. They differ, however, a little from those agglutinins that occur in the tribe Genisteae. Concerning the agglutinins of Lotus tetragonolobus this was demonstrated by Morgan and Watkins (108) and Krüpe (84) by means of their agglutination-inhibition tests. It will be demonstrated in Chapter III of this paper also with regard of L. siliquosus.

A multiplicity of genera and great variations in agglutinin content obtain in the tribe Galegeae. There are several large genera that seem to be totally devoid of agglutinins, e.g. Astragalus, Indigofera and Oxytropis. The occurrence of agglutinins is almost the rule in the seeds of the genera Caragana, Galega, Halimodendron, Robinia and Wistaria. The specificity of most of the agglutinins is low, they agglutinate the red cells of most studied animals, and do not agglutinate human red cells selectively. The one sample of Caragana frutex var. latifolia examined by the author was an exception by showing anti-A+B specificity. The author received two samples of this species from the same garden in two consecutive years and was able to demonstrate similar specificity in each. The seeds were sown and the genus was verified, but the species could not be identified with certainty.

The tribe Hedysareae seems to be poor in agglutinins. Several of the examined samples of Coronilla varia contained an agglutinin of the type anti-A+B, which reacted also with the red cells of rabbit (and hog (84)). The rest of the agglutinins of the Hedysareae are weak and sporadic.

Of the tribe Dalbergieae, the seeds of Andira inermis appear to contain an anti-guinea pig, those of Lonchocarpus discolor an anti-rabbit+guinea pig, agglutinin.

With regard to the occurrence of agglutinins, the tribe Vicieae shows striking homogeneity. With the exception of the genus Cicer, nearly all seeds of this tribe seem to contain an agglutinin that reacts with the red cells of man, rabbit and guinea pig

preferentially, showing no blood group specificity. This rule admits of only few exceptions: there are some seed samples that do not seem to contain agglutinins, there are a few others containing anti-A agglutinins, and still others containing anti-N agglutinins.

The occurrence of agglutinins in the tribe Phaseoleae shows greater variety. Agglutinins occur, as a rule, in the genera Canavalia, Dolichos, Erythrina, Glycine, Phaseolus and Vigna, but they are not at all identical. Good anti-A reagents are frequent in the seeds of Dolichos biflorus, Phaseolus lunatus and Amphicarpaea species.

The present author believes that conclusions can be based on Table 5 regarding the rules that govern the incidence of seed agglutinins in the family Leguminosae. Admittedly, the author's material does not consist of random samples. Since one of the aims never lost sight of in the course of the work was to find potential reagents for the use of the serologist, such species are represented by numerous samples as were considered to be the most promising probables. Efforts were made, on the other hand, in order that as many species as possible might be represented by at least one seed sample. The material of Table 5 is not very large, either. It comprises less than 10 % of the 12 000 species and about 30 % of the 600 genera that belong to the family Leguminosae, the third largest of the flowering plants.

At any rate this material seems adequate to prove that the occurrence of agglutinins is governed by certain rules. The occurrence can perhaps be said to conform to the taxonomic plant system to some extent though by no means absolutely. Proof of this are, in particular, the total absence of agglutinins in the seeds of certain tribes, e.g. Trifolieae, and the almost regular presence in the seeds of Vicieae. This fact was observed by Renkonen (122) already, but Tétry et coll. (141) suggested that the occurrence or nonoccurrence of agglutinins was the property of a given species, not of a genus.

III SEROLOGICAL SPECIFICITY OF PLANT AGGLUTININS

Section one: SPECIFIC AGGLUTINATION AND PRECIPITATION

SURVEY OF LITERATURE

1. SPECIFIC AGGLUTINATION

Species specificity

Most of the plant agglutinins examined react with the red cells of different animal species more or less selectively. The agglutinins of different plants prefer different animal species. For this reason they doubtless are valuable tools in the study of the similarities and differences between animal red cell receptors. Many of the studies on this subject thus far have had a couple of weak points. First, several investigators have not carried out quantitative studies. They have contented themselves with saying that a given plant extract agglutinates the red cells of an animal but not those of another. If it so happens that, other things being equal, the extract of another worker is ten times more active, he may find that the extract agglutinates the red cells of both animal species. Still another investigator may full well find his extract from the same plant species inactive towards the red cells of both animal species. If all three investigators had titrated their extracts, the results would not have been paradoxical.

Another weak point of many works is the fact that an animal species has been represented by but one individual. It is possible that there are plant agglutinins showing animal blood group specificity. In fact, differences have been found to exist between various individuals of certain animal species regarding the reactivity of the red cells with a given plant extract (36).

Bird (12) and Ottensooser (115, 116) have employed several individuals of each animal species in studying plant agglutinins. Bird studied the effect of 12 seed extracts on the red cells of

guinea pig, rabbit, pigeon, chicken, dog, horse, buffalo, cow, goat, sheep, and man (A and O). He did not notice appreciable differences between different individuals of a species. He did not titrate the extracts. With the aid of the twelve plant extracts he was able to differentiate between the erythrocytes of most of the animals. Bird mentions the possible usability of plant agglutinins in forensic pathology.

Ottensooser used the seed extracts of 12 different Vicia species together with the red cells of rabbit, horse, mouse, pig, man, (A_1, B, O), rat, Java monkey, chimpanzee, chicken, sheep, and goat. He also failed to titrate the extracts. He intimates that plant agglutinins might be useful as auxiliary tools to medical jurisprudence.

Krüpe (79) studied an extensive series of seed extracts using the cells of man (A_1, A_2, B, O), sheep, rabbit, guinea pig, cow, and hog. He did not employ several specimens of an animal species, but he carried out titrations, too. His table shows among other things that most seeds of the tribe Vicieae contain an agglutinin strongly reactive with the examined individuals of man, rabbit and guinea pig. There are plant extracts, too, that react with the red cells of rabbit only, and, as Krüpe found out later, with those of hog, if more weakly (84). Such extracts were prepared from Coronilla varia, Sophora japonica, Glycine soja and Sarothamnus scoparius.

Blood group specificity

So far as the present author knows, there have been only two attempts to find plant agglutinins that might detect blood groups in species other than Homo sapiens. Dujarric de la Rivière et coll. (36) studied the effect of the agglutinin of Phaseolus vulgaris on the erythrocytes of 40 human beings, besides 101 cows, 56 horses, 15 mules, 32 sheep, and 20 goats. They did not notice positive differences between the individuals of any one species with the exception of the cows. The cells of some of these produced a negative reaction, while the titres of the others varied between 1/1 and 1/64. The workers believed that the red cells of cows were of two types, which were differentiable by potato extract, too.

Levine et coll. (94) found that the red cells of all the 13 chimpanzees examined were agglutinated by several absorbed rabbit anti-M sera, while the red cells of only 4 chimpanzees gave positive reactions with extracts of Vicia graminea known to contain an anti-N agglutinin. On the basis of their agglutination and absorption tests they classified 9 of the chimpanzees as M and 4 as MN. The red cells of the MN chimpanzees did not react with rabbit anti-N sera, however. According to their opinion, the human and chimpanzee M have more in common than do human and chimpanzee N.

Investigations into plant agglutinins that differentiate between human beings are so much more numerous. Several agglutinins of this kind have been discovered. No matter how disparate the plants that yield them, and much as they may differ serologically, nearly all the agglutinins have a property in common. Apart from a few exceptions (114, 99) their specific receptor in human red cells is related to one or some of the ABO agglutinogens.

Table 5 includes most known plant agglutinins that have been found to possess blood group specificity. A most thorough treatment of this subject is found in the monograph of Krüpe (84).

It has been commonly observed that ABO blood group specific agglutinins from various plant species are generally different each. Three anti-A agglutinins from Dolichos biflorus, Phaseolus lunatus and Vicia cracca will serve as an example. Dolichos biflorus agglutinates only cells containing the agglutinogen A, and A_1 cells much better than it does A_2 cells. Likewise the agglutinin of Phaseolus lunatus is a pretty pure anti-A, still it strongly agglutinates A_2 cells, too. Although the extracts from Vicia cracca, too, are good anti-A reagents, they agglutinate also O and B cells.

This fact and the extreme rareness, if not nonexistence, of fully blood group specific plant agglutinins is easily accounted for by their origin, in the opinion of Boyd and Shapleigh (25). It must be assumed that a certain plant agglutinin *accidentally* possesses a configuration that is complementary to the chemical groupings of a blood group substance. Since human blood group substances are very closely related, chemically (107), it stands to reason of course that this agglutinin is more or less complemen-

tary to one or some of the other human blood group substances, too.

Morgan and Watkins (108) found that the anti-A and anti-B agglutinins of Sophora japonica are inseparable. They considered the possible relationship of this agglutinin to the hypothetical blood group factor C, which is believed to be common to the red cells of groups A and B but absent from O (146).

Bird (17) found that the seed extracts from Crotalaria striata also agglutinated A and B cells. The anti-A and anti-B agglutinins were inseparable. The activity of the anti-A was stronger unlike in the case of Sophora japonica. Bird does not believe that of two different plant agglutinins both could possibly be anti-C. In his opinion the agglutinins of Crotalaria striata are better adapted to the structure A and Sophora japonica agglutinins to the structure B. He does not think it necessary to presume the existence of a third factor. Thus his view resembles that of Boyd and Shapleigh described above.

Krüpe (84) believes that blood group specific plant agglutinins react with the same receptors as isoagglutinins. He bases this opinion on his observations that red cells saturated with plant anti-A agglutinin absorb neither animal nor vegetable anti-A, and thinks that the same is true of the rest of plant agglutinins respectively.

Bird (7) has shown that the receptor of Dolichos biflorus agglutinin is not the Forssman antigen. The extract from Dolichos weakly agglutinates the red cells of sheep, it is true, but it does not hemolyse them in the presence of complement and neither does it agglutinate the cells of chicken.

Bird (13) has studied the effect of plant agglutinins on red cells, the T receptor of which had been unmasked by a bacterial contamination. He found that Phaseolus lunatus extract containing an anti-A agglutinin agglutinated O cells, too, if these had been «transformed» in like manner. Dolichos biflorus extract, on the contrary, did not agglutinate transformed O cells. Bird concluded that some seed extracts are capable of detecting the T receptor, while others are not. Krüpe and Dötzer (85) did not find a single T agglutinin in the 10 different seed extracts they studied, among them a Phaseolus lunatus extract. A few blood group specific plant agglutinins admittedly lost

their specificity if the test cells had been transformed with the T ferment. Inhibition tests by blood group substances and simple sugars showed, however, that the unspecific reactions had not been caused by the T agglutinin. Agglutination of the transformed cells by plant agglutinins was inhibited by blood group substances and certain simple sugars, while agglutination by normal human serum was not. It was found that Lotus tetragonolobus agglutinin and eel serum divided A_1 cells treated with the T ferment into two groups according as they reacted positively or negatively. A similar dichotomy was noticed in the A_1B group. The authors believe that the property is hereditary and hardly connected with the recognised blood groups. Agglutination by Lotus tetragonolobus extract of the positively reacting A_1 cells treated with the T ferment was inhibited by H substance and L-fucose. This led the authors to the conclusion that the T ferment is capable of detecting other receptors, too, in addition to the T receptor.

In 1953 Ottensooser and Silberschmidt (114) found the first vegetable anti-N. Krüpe (84) confirmed their observation. Because anti-N agglutinin was obtainable by elution from human and chimpanzee M and MN cells, but not from human N cells (94), the anti-N specificity of this seed agglutinin is probably a little short of absolute. In the seeds of some Bauhinia species the present author has found an agglutinin of low anti-N specificity (99).

Usability of plant agglutinins in routine serology

Blood group specific plant agglutinins have been known for nearly ten years. During this period they have been widely used as reagents in routine blood grouping, too. Opinions differ as to the range of their usability but the consensus is probably in favour of their use for certain purposes.

Koulumies (69, 70) was the first to study the usability of the agglutinins of Vicia cracca and Cytisus sessilifolius in A_1—A_2 and A_1B—A_2B subgrouping. He came to the conclusion that, as an anti-A_1 reagent, Vicia cracca equals absorbed B sera; likewise, the anti-H of Cytisus sessilifolius is as good as that of

guinea pig, rabbit, pigeon, chicken, dog, horse, buffalo, cow, goat, sheep, and man (A and O). He did not notice appreciable differences between different individuals of a species. He did not titrate the extracts. With the aid of the twelve plant extracts he was able to differentiate between the erythrocytes of most of the animals. Bird mentions the possible usability of plant agglutinins in forensic pathology.

Ottensooser used the seed extracts of 12 different Vicia species together with the red cells of rabbit, horse, mouse, pig, man, (A_1, B, O), rat, Java monkey, chimpanzee, chicken, sheep, and goat. He also failed to titrate the extracts. He intimates that plant agglutinins might be useful as auxiliary tools to medical jurisprudence.

Krüpe (79) studied an extensive series of seed extracts using the cells of man (A_1, A_2, B, O), sheep, rabbit, guinea pig, cow, and hog. He did not employ several specimens of an animal species, but he carried out titrations, too. His table shows among other things that most seeds of the tribe Vicieae contain an agglutinin strongly reactive with the examined individuals of man, rabbit and guinea pig. There are plant extracts, too, that react with the red cells of rabbit only, and, as Krüpe found out later, with those of hog, if more weakly (84). Such extracts were prepared from Coronilla varia, Sophora japonica, Glycine soja and Sarothamnus scoparius.

Blood group specificity

So far as the present author knows, there have been only two attempts to find plant agglutinins that might detect blood groups in species other than Homo sapiens. Dujarric de la Rivière et coll. (36) studied the effect of the agglutinin of Phaseolus vulgaris on the erythrocytes of 40 human beings, besides 101 cows, 56 horses, 15 mules, 32 sheep, and 20 goats. They did not notice positive differences between the individuals of any one species with the exception of the cows. The cells of some of these produced a negative reaction, while the titres of the others varied between 1/1 and 1/64. The workers believed that the red cells of cows were of two types, which were differentiable by potato extract, too.

Levine et coll. (94) found that the red cells of all the 13 chimpanzees examined were agglutinated by several absorbed rabbit anti-M sera, while the red cells of only 4 chimpanzees gave positive reactions with extracts of Vicia graminea known to contain an anti-N agglutinin. On the basis of their agglutination and absorption tests they classified 9 of the chimpanzees as M and 4 as MN. The red cells of the MN chimpanzees did not react with rabbit anti-N sera, however. According to their opinion, the human and chimpanzee M have more in common than do human and chimpanzee N.

Investigations into plant agglutinins that differentiate between human beings are so much more numerous. Several agglutinins of this kind have been discovered. No matter how disparate the plants that yield them, and much as they may differ serologically, nearly all the agglutinins have a property in common. Apart from a few exceptions (114, 99) their specific receptor in human red cells is related to one or some of the ABO agglutinogens.

Table 5 includes most known plant agglutinins that have been found to possess blood group specificity. A most thorough treatment of this subject is found in the monograph of Krüpe (84).

It has been commonly observed that ABO blood group specific agglutinins from various plant species are generally different each. Three anti-A agglutinins from Dolichos biflorus, Phaseolus lunatus and Vicia cracca will serve as an example. Dolichos biflorus agglutinates only cells containing the agglutinogen A, and A_1 cells much better than it does A_2 cells. Likewise the agglutinin of Phaseolus lunatus is a pretty pure anti-A, still it strongly agglutinates A_2 cells, too. Although the extracts from Vicia cracca, too, are good anti-A reagents, they agglutinate also O and B cells.

This fact and the extreme rareness, if not nonexistence, of fully blood group specific plant agglutinins is easily accounted for by their origin, in the opinion of Boyd and Shapleigh (25). It must be assumed that a certain plant agglutinin *accidentally* possesses a configuration that is complementary to the chemical groupings of a blood group substance. Since human blood group substances are very closely related, chemically (107), it stands to reason of course that this agglutinin is more or less complemen-

tary to one or some of the other human blood group substances, too.

Morgan and Watkins (108) found that the anti-A and anti-B agglutinins of Sophora japonica are inseparable. They considered the possible relationship of this agglutinin to the hypothetical blood group factor C, which is believed to be common to the red cells of groups A and B but absent from O (146).

Bird (17) found that the seed extracts from Crotalaria striata also agglutinated A and B cells. The anti-A and anti-B agglutinins were inseparable. The activity of the anti-A was stronger unlike in the case of Sophora japonica. Bird does not believe that of two different plant agglutinins both could possibly be anti-C. In his opinion the agglutinins of Crotalaria striata are better adapted to the structure A and Sophora japonica agglutinins to the structure B. He does not think it necessary to presume the existence of a third factor. Thus his view resembles that of Boyd and Shapleigh described above.

Krüpe (84) believes that blood group specific plant agglutinins react with the same receptors as isoagglutinins. He bases this opinion on his observations that red cells saturated with plant anti-A agglutinin absorb neither animal nor vegetable anti-A, and thinks that the same is true of the rest of plant agglutinins respectively.

Bird (7) has shown that the receptor of Dolichos biflorus agglutinin is not the Forssman antigen. The extract from Dolichos weakly agglutinates the red cells of sheep, it is true, but it does not hemolyse them in the presence of complement and neither does it agglutinate the cells of chicken.

Bird (13) has studied the effect of plant agglutinins on red cells, the T receptor of which had been unmasked by a bacterial contamination. He found that Phaseolus lunatus extract containing an anti-A agglutinin agglutinated O cells, too, if these had been «transformed» in like manner. Dolichos biflorus extract, on the contrary, did not agglutinate transformed O cells. Bird concluded that some seed extracts are capable of detecting the T receptor, while others are not. Krüpe and Dötzer (85) did not find a single T agglutinin in the 10 different seed extracts they studied, among them a Phaseolus lunatus extract. A few blood group specific plant agglutinins admittedly lost

their specificity if the test cells had been transformed with the T ferment. Inhibition tests by blood group substances and simple sugars showed, however, that the unspecific reactions had not been caused by the T agglutinin. Agglutination of the transformed cells by plant agglutinins was inhibited by blood group substances and certain simple sugars, while agglutination by normal human serum was not. It was found that Lotus tetragonolobus agglutinin and eel serum divided A_1 cells treated with the T ferment into two groups according as they reacted positively or negatively. A similar dichotomy was noticed in the A_1B group. The authors believe that the property is hereditary and hardly connected with the recognised blood groups. Agglutination by Lotus tetragonolobus extract of the positively reacting A_1 cells treated with the T ferment was inhibited by H substance and L-fucose. This led the authors to the conclusion that the T ferment is capable of detecting other receptors, too, in addition to the T receptor.

In 1953 Ottensooser and Silberschmidt (114) found the first vegetable anti-N. Krüpe (84) confirmed their observation. Because anti-N agglutinin was obtainable by elution from human and chimpanzee M and MN cells, but not from human N cells (94), the anti-N specificity of this seed agglutinin is probably a little short of absolute. In the seeds of some Bauhinia species the present author has found an agglutinin of low anti-N specificity (99).

Usability of plant agglutinins in routine serology

Blood group specific plant agglutinins have been known for nearly ten years. During this period they have been widely used as reagents in routine blood grouping, too. Opinions differ as to the range of their usability but the consensus is probably in favour of their use for certain purposes.

Koulumies (69, 70) was the first to study the usability of the agglutinins of Vicia cracca and Cytisus sessilifolius in A_1—A_2 and A_1B—A_2B subgrouping. He came to the conclusion that, as an anti-A_1 reagent, Vicia cracca equals absorbed B sera; likewise, the anti-H of Cytisus sessilifolius is as good as that of

bovine serum. His Cytisus sessilifolius extract did not agglutinate A_2B cells, nor did bovine anti-H, either.

Subsequently several reports have been published to the effect that good results have been secured by means of plant agglutinins in the diagnosis of the subgroups of blood groups A and AB. Bird (8) has found the extract of Dolichos biflorus to be a most reliable anti-A_1 reagent. Boyd and Shapleigh (26) have obtained good results using the extracts of Dolichos biflorus and Ulex europaeus. Herrman (56) has carried out several thousands of subgroupings with the aid of Laburnum alpinum and Phaseolus limensis. The widest experience in this subject has probably been gained by Krüpe (78, 84), who, since 1949, has used several plant extracts besides isoagglutinins in all his blood group tests. As many as about 10 000 blood samples have thus been tested. Krüpe finds these reagents excellent in A subgroup diagnosis.

Opinions of the suitability of plant agglutinins for general ABO blood group diagnosis are at variance. Bird (6, 10) regards vegetable anti-A agglutinins as suitable anti-A reagents in certain circumstances. At the same time he complains of the absence of a dependable vegetable anti-B. Dunsford and Hutchison (37) have found that the extracts of Dolichos biflorus and Phaseolus lunatus do not agglutinate A_3B and A_4 cells, and do not consider the extracts to be suitable anti-A reagents. Kahl (64) determined the ABO groups of 500 blood samples employing the personnel of an average hospital laboratory. He used both the normal method and 4 plant extracts (Laburnum alpinum, Phaseolus limensis, Vicia cracca, Sophora japonica). In 2 % of the cases he obtained erroneous results with the plant extracts, and these were due mainly to the absence of a good anti-B.

Vegetable anti-H agglutinins have turned out to be good reagents in the separation of the individuals of any blood group into secretors and nonsecretors (71, 24, 135). The vegetable anti-N has probably been little used in routine work.

2. SPECIFIC PRECIPITATION

Boyd and Shapleigh (25, 27) have shown that human blood group substances are specifically precipitated by some blood

group specific plant agglutinins. The extract of Phaseolus limensis, for example, caused precipitation in the salivas of secretors belonging to blood groups A_1 and A_2, but not in the salivas of the secretors of blood group O, nor in the salivas of the nonsecretors of any blood group. The seed extract also precipitated with the A substance made from the gastric mucin of hog. In a later report (29) it was stated that 30 % of the protein of the refined Phaseolus preparate precipitated with A substance. The results of a quantitative study were presented showing a precipitin curve very similar to that plotted for the reactions of A substance with human anti-A and presented by Kabat (63). The quantity of the precipitate increased as the amount of A substance was raised until a certain maximum was reached; an excess of A substance was accompanied with a decrease in the amount of the precipitate. The plant agglutinin appeared to possess lower affinity for A substance than did human anti-A.

OWN INVESTIGATIONS INTO SPECIFIC AGGLUTINATION

I. SPECIES SPECIFICITY

In the following the results of Table 5 will be treated from the point of view of species specificity. As the table shows, most of the seed extracts tested by the present author seem to possess at least traces of species specificity. The results do not perhaps allow one to draw positive conclusions since each animal species is represented by one individual only. As the results, however, generally agree with the findings of earlier investigators, they are likely to have certain significance.

It seems that bovine cells are poorly agglutinated by most seed extracts, while on the other hand the titres of many extracts against the red cells of man, rabbit and guinea pig are high.

There are plant agglutinins that react only or preferentially with the red cells of but one or two of the species studied. For example the seeds of a few Tephrosia and Andira species appear to contain anti-guinea pig agglutinins, and those of certain Parkia species anti-rabbit+guinea pig agglutinins. It seems further that many human blood group specific agglutinins are species specific, too, i.e. they agglutinate poorly the red cells of other species.

It is not possible to base conclusions on the author's experiments as to the blood group specificity of plant agglutinins in animals.

In this investigation several seed extracts were found capable of selective agglutination of human erythrocytes as shown in the principal Table 5 in the preceding chapter. The specificity often manifested itself no matter which method was used in the test. In some cases, however, anti-H agglutinins were demonstrable only by the papain and/or kollidon method, obviously on account of their weakness. That this is not due to qualitative differences is shown by the fact that as soon as some of these weak agglutinins had been concentrated, they reacted also in saline milieu (101). Not a single such extract was found as would agglutinate different human red cells unspecifically by the saline or AB serum method while showing selectivity by the papain or kollidon method. In theory this would be possible because the titres were usually higher by the latter methods.

As stated in Chapter II, all plant extracts that agglutinated human red cells selectively in the first tests were subjected to further study. These will be described in this chapter.

Methods

New extracts were prepared from all seeds. The titrations were carried out in the same way as the tests of Chapter II. Because additional information of interest was yielded only infrequently by tests with enzyme treated cells and intact cells in kollidon milieu, routine use of these tests was not made in the additional studies.

The seed extracts were titrated at first using the red cells of 8 persons. They had been selected so that they represented the blood groups A_1, A_2, A_1B, A_2B, B, OO, MM, MN, NN, S, ss, P, pp, Le(a+b—), Le(a—b+), Rh+ and Rh—. If the results were in agreement with those of previous investigators, they were deemed reliable without further tests being undertaken. If the results did not agree or if a blood group specific agglutinin, unknown until then, was found, more blood samples, sometimes several hundred, were resorted to in the investigation. At this stage only the blood groups of ABO and MN systems were known.

Results

As will be seen from Table 5, the first series of tests on the extracts produced only two groups of blood group specific

agglutinins: such as showed an affinity for one or some of the agglutinogens of the ABO system, and such as seemed to possess a kind of anti-N specificity. These observations were confirmed by additional investigations.

By the year 1956 approx. 40 plant species had been reported to contain ABO specific agglutinins occurring in the seeds (84, 116, 17, 99). The present author was able to verify earlier observations in the cases of 23 plant species. In addition to this the author discovered 46 new Leguminosae species, the seeds of which contained ABO specific agglutinins.

Table 6 presents the species that have been found to contain ABO specific agglutinins, in the present investigation. According to their specificity they are divided into four groups: anti-A, anti-B, anti-H, and anti-A+B. It does not follow that e.g. anti-A extracts do not agglutinate also cells other than A ones. Fundamentally, the division is artificial. In fact, most «blood group specific» plant agglutinins appear to agglutinate all human red cells, provided that their concentration is high. The differences between agglutination titres, however, can be quite considerable. In the group anti-A, for example, the present author accordingly included those extracts which in his opinion can be used as anti-A reagents if necessary. The requirement to be met by an extract was that its titre against A_2 cells had to be higher by at least 3 or 4 powers of two than it was against B or O cells.

The term anti-A+B denotes that the extract agglutinates red cells that contain either the agglutinogen A or B, but that it agglutinates cells in which both are lacking much more weakly. The letters A and B are in alphabetical order, the group includes such agglutinins as react with A cells more strongly, such as react more strongly with B cells and also such as yield nearly identical titres with both kinds of cells.

Several of the blood group specific extracts were titrated also by the papain and kollidon methods using A_1, B and O cells. The results are given in Table 7. A closer study of the table will show that if the specificity of an extract manifests itself by the saline method, it usually manifests itself no matter which of the four methods is used. In kollidon milieu the degree of specificity often becomes a little lower, while papainised cells seem to be

TABLE 6

A list of Leguminosae species whose seeds were found to contain agglutinins showing some kind of ABO blood group specificity

Explanatory notes: w=weak agglutination by undiluted extract; h=hemolysis

For synonyms of the names for the species and for fuller explanations see Table 5.

	No. of seed samples tested	No. of samples showing specificity	Titres against different kinds of red cells in different media																Confirming results (ref. no.)
			saline milieu						serum milieu						saline milieu				
			A_1	A_2	A_1B	A_2B	B	O	A_1	A_2	A_1B	A_2B	B	O	cow	sheep	rabbit	g-pig	chick
anti-A extracts																			
Lathyrus pratensis	4	1	4	4	4	4	4	4	256	64	256	8	2	—	—	—	64	32	—	
Vicia cracca	27	21	1024	128	512	16	8	4	8192	1024	2048	128	64	32	—	—	512	256	—	122, 30, 79, 84
— dumetorum	3	3	8	8	8	8	8	8	64	8	64	4	—	—	—	—	64	32	—	
— peregrina	7	6	2	2	2	2	2	2	64	8	32	4	—	—	—	—	16	4	—	115, 116
— tenuifolia	4	2	64	32	32	16	8	4	32	8	16	8	—	—	—	—	256	64	—	30
— varia	2	2	8	4	4	8	2	1	32	4	16	—	—	—	—	—	256	128	—	
— villosa	7	7	64	2	32	—	—	—	1024	32	512	2	—	—	—	—	128	64	—	65, 79
Amphicarpaea bracteata	1	1	8	—	4	—	—	—	64	16	64	—	—	—	—	—	—	—	—	99
— Edgeworthii	1	1	16	—	4	—	—	—	64	16	64	—	—	—	—	—	—	—	—	99
Dolichos biflorus	2	2	256	2	64	—	—	—	1024	16	512	1	—	—	—	—	—	—	—	5, 30, 84
Phaseolus lunatus	2	1	1024	64	512	16	1	—	4096	64	1024	16	—	—	—	—	—	—	—	20, 21, 65, 84
anti-A+B extracts																			
Crotalaria compista	1	1	8	2	8	1	1	—	64	8	64	8	8	—	—	—	—	—	—	
— striata	2	2	32	4	32	2	—	—	512	16	256	32	8	2	—	—	—	—	—	17, 144
— usaramoensis	2	2	128	8	128	32	4	—	1024	32	1024	32	8	2	—	—	64	—	—	

—	striata	2	2	32	4	32	2	—	—	512	16	256	32	8	2	—	—	—	—	—	17, 144		
—	usaramoensis	2	2	128	8	128	32	4	—	1024	32	1024	32	8	2	—	—	64	—	—			

			A_1	A_2	A_1B	A_2B	B	O	A_1	A_2	A_1B	A_2B	B	O	cow	sheep	rabbit	g-pig	chick	
Caragana frutex v. latifolia	1	1	—	—	—	—	—	—	128	8	128	128	128	—	—	256	—	2	—	
Dolichos lablab	5	3	128	64	64	64	64	64	16	4	32	64	64	—	—	32	512	512	32	
Vigna catjang	1	1	64	64	64	64	64	64	64	8	128	128	128	1	—	w	128	128	8	
Sophora japonica	4	4	—	—	—	—	—	—	256	16	1024	1024	2048	2	—	—	256	8	—	77, 84
Coronilla varia	7	7	—	—	—	—	—	—	8	—	32	32	64	—	—	—	16	—	—	77, 84
Bandeiraea simplicifolia (recently harvested seeds)	1	1	8	2	16	128	128	—	32	2	256	256	512	—						
anti-B extract																				
Bandeiraea simplicifolia (one year old seeds)	1	1	—	—	4	32	64	—	—	—	16	256	256	—	64	—	2048	h	—	99
anti-H extracts																				
Virgilia divaricata[1]	1	1	—	—	—	—	—	—	—	—	—	—	—	—	8	16	—	1	8	99
— lutea	1	1	—	2	—	—	1	2	—	2	—	1	—	4	—	—	—	—	—	99
— oroboides	1	1	—	4	—	—	1	4	—	4	—	1	2	8	2	2	—	—	2	99
Cytisus argenteus	1	1	2	32	—	8	32	64	2	32	—	8	32	64	—	—	—	—	—	30
— benehoavensis	1	1	—	2	—	—	2	4	—	4	—	—	2	4	—	—	—	—	—	
— biflorus	3	3	—	—	—	—	—	1	—	8	—	1	4	8	—	—	4	—	—	122, 79
— capitatus	2	2	—	—	—	—	—	—	—	4	—	2	4	8	—	—	16	—	—	.
— caucasicus	1	1	—	1	—	—	1	2	—	1	—	—	1	2						
— lusitanicus	1	1	2	8	1	4	8	32	2	8	1	4	8	16	—	—	32	—	—	
— nigricans	3	2	—	4	—	—	1	8	—	4	—	—	1	8	—	—	—	—	—	79
— nigricans v. elongatus	1	1	—	1	—	—	1	1	—	2	—	—	2	4						
— osmarensis	1	1	—	2	—	1	2	4	—	2	—	—	2	4	—	—	—	—	—	
— praecox	1	1	—	2	—	—	1	4	—	2	—	—	1	8	—	—	—	—	—	122, 79
— purpureus	1	1	—	—	—	—	—	—	—	1	—	—	—	2	—	—	—	—	—	79
— sessilifolius	10	10	—	16	—	—	4	32	—	16	—	—	4	64	—	—	—	—	—	122, 79
— supinus	2	2	—	—	—	—	—	—	—	—	—	—	—	2	—	—	4	—	—	
— Weldeni[1]	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Genista ancistrocarpa	1	1	—	1	—	—	1	2	—	2	—	—	2	2						
— anglica	1	1	—	1	—	—	1	4	—	2	—	—	2	2						(cont'd)

[1] See Table 7.

TABLE 6 (cont'd)

	No. of seed samples tested	No. of samples showing specificity	Titres against different kinds of red cells in different media																Confirming results (ref. no.)
			saline milieu						serum milieu						saline milieu				
			A_1	A_2	A_1B	A_2B	B	O	A_1	A_2	A_1B	A_2B	B	O	cow	sheep	rabbit	g-pig	chick
Genista aristata	1	1	—	4	—	—	2	4	—	2	—	—	2	4						30
— clavata	1	1	2	16	—	8	16	32	2	16	—	8	16	64	—	—	—	—	—	
— germanica[1]	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
— horrida	1	1	—	4	—	—	2	8	—	4	—	—	2	8						
— monosperma	5	5	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	
— sagittalis	11	11	—	16	—	—	4	16	—	16	—	—	4	32	—	—	—	—	—	21, 79
— silvestris v. dalmatica	1	1	—	8	—	—	4	16	—	16	—	—	8	32						
— sphaerocarpa	2	2	—	—	—	—	—	2	—	1	—	—	1	4	—	—	—	—	—	
— triacanthos	1	1	1	32	—	4	32	64	1	32	—	4	32	64						
— umbellata	1	1	—	16	—	2	4	16	—	8	—	4	8	16						
Laburnum alpinum	4	4	—	16	—	2	2	16	—	16	—	2	4	32	—	—	—	—	—	122, 75, 79
— anagyroides	1	1	—	—	—	—	—	1	—	1	—	—	—	4						75, 79
— Watereri	3	3	—	4	—	1	4	8	—	8	—	2	8	32	—	—	—	—	—	
Petteria ramentacea	4	4	—	4	—	—	4	8	—	8	—	—	8	8	—	—	—	—	—	
Ulex densus	1	1	—	4	—	1	2	4	1	4	—	1	2	16						
— europaeus	4	4	1	16	—	4	8	16	1	16	—	8	16	64	—	—	—	—	—	30, 79
— Gallii	1	1	—	4	—	1	4	8	1	16	—	4	8	32						
— Jussiaei	1	1	—	4	—	1	4	8	1	8	—	2	4	16						30
— nanus	1	1	2	8	—	4	8	32	1	64	—	8	32	64						
Lotus biflorus	1	1	—	4	—	2	2	16	1	16	—	4	8	32						
— Requieni	2	1	—	4	—	—	—	8	—	16	—	—	—	32						
— siliquosus	4	4	—	2	—	—	—	4	—	8	—	—	4	16	—	—	—	—	—	
— tetragonolobus	29	29	—	8	—	—	4	16	—	16	—	—	8	32	—	—	—	—	—	122, 75, 30, 79

[1] See Table 7.

TABLE 7

Titres of some ABO-specific seed extracts determined by various methods.

(For synonyms of the names for the species see Table 5)

	saline medium			AB serum medium			papain treated cells in saline			3.6 % kollidon medium		
	blood group of the test cells											
	A_1	B	O	A_1	B	O	A_1	B	O	A_1	B	O
Vicia dumetorum	8	8	8	64	—	—	256	256	256	32	32	32
— peregrina	2	2	2	64	—	—	32	32	32	8	8	8
Amphicarpaea Edgeworthii	16	—	—	64	—	—	512	—	—	256	—	—
Dolichos biflorus	256	—	—	1024	—	—	4096	—	—	4096	—	—
Phaseolus lunatus	1024	1	—	4096	—	—	8192	8	—	2048	2	—
Crotalaria striata	32	—	—	512	8	2	1024	16	4	1024	32	8
— usaramoensis	128	4	—	1024	8	2	1024	8	4	512	16	4
Caragana frutex	—	—	—	128	128	—	64	64	—	32	16	—
Sophora japonica	—	—	—	256	2048	2	16	64	—	4	8	1
Coronilla varia	—	—	—	8	64	—	8	32	—	1	2	—
Virgilia divaricata	—	—	—	—	—	—	—	—	2	—	—	8
— oroboides	—	1	4	—	2	8	—	4	16	4	16	64
Cytisus lusitanicus	2	8	32	2	8	16	32	64	64	32	64	64
— sessilifolius	—	4	32	—	4	64	—	16	128	1	4	128
— Weldeni	—	—	—	—	—	—	—	—	4	1	2	8
Genista ancistrocarpa	—	1	2	—	2	2	—	4	8	1	2	4
— germanica	—	—	—	—	—	—	—	1	4	4	8	8
— monosperma	—	—	—	—	—	2	—	1	4	1	2	4
— sagittalis	—	4	16	—	4	32	8	64	512	4	32	128
Laburnum alpinum	—	2	16	—	4	32	—	16	64	—	8	32
Petteria ramentacea	—	4	8	—	8	8	—	16	32	1	16	64
Ulex europaeus	1	8	16	1	16	64	64	128	256	16	128	256
Lotus tetragonolobus	—	4	16	—	8	32	—	32	128	—	16	64

agglutinated by nearly all of the tested extracts as selectively as intact cells are agglutinated in NaCl milieu.

Three of the examined plant extracts contained agglutinins of the type anti-A+B which reacted in serum but not in saline milieu. The species were Caragana frutex var. latifolia, Sophora japonica and Coronilla varia. These all reacted more poorly

when tested by the papain and kollidon methods, the reactions were nevertheless specific.

There were also two Vicia species, V. dumetorum and V. peregrina, whose seed extracts agglutinated human erythrocytes unspecifically in saline milieu as Vicia extracts generally do. In AB serum milieu they anyway acted as anti-A reagents. These two extracts agglutinated human red cells unspecifically when tested by the papain and kollidon methods. The extracts of Vigna catjang and Dolichos lablab behaved in a corresponding manner, except that their specificity was of the type anti-A+B.

Another group of blood group specific plant agglutinins, the anti-N agglutinins, is small. Only two species belonging to this group had been found previous to the present investigation (114, 99). The author confirmed these findings and discovered 6 more plant species that contain agglutinins of this type. All these 8 species are presented in Table 8. Of these four belong to the tribe Vicieae and 4 to the genus Bauhinia. The specificity of the anti-N agglutinin of Vicia graminea is decidedly superior to that of the other species. As a matter of fact, many Bauhinia extracts, other than those included in the table, seemed to possess slight anti-N affinity. The agglutinins of only the four species presented in Table 8 exhibited an affinity distinct enough to warrant their classification as anti-N. Even they are hardly usable in routine work.

Anti-N specificity was not demonstrable by the papain or kollidon method. Anti-N specific reactions could be elicited from the Bauhinia extracts in AB serum milieu only, they were inactive in saline milieu. The extracts of Lens nigricans, Vicia picta and Vicia leganyana agglutinated human red cells unspecifically in saline milieu like nearly all of the seed extracts of the tribe Vicieae studied by the author. While Vicia extracts were, as a rule, almost inactive in AB serum milieu, the extracts of the above three species acted as anti-N reagents in this milieu. The agglutinin of the seeds of Vicia graminea behaved in a contrary manner. It agglutinated red cells selectively in saline milieu, and unspecifically in AB serum. Similar observations on the behaviour of the agglutinins of Vicia graminea have been recorded by Krüpe (84).

TABLE 8

Titres of the anti-N specific seed extracts against different kinds of red cells tested by various methods

	No. of seed samples tested	No. of samples showing specificity	saline medium			AB serum medium			papain treated cells in saline			3.6 % kollidon medium			saline medium					Confirming results (ref. no.)
			test cells, human red cells of blood group O												red cells of					
			M	MN	N	M	MN	N	M	MN	N	M	MN	N	cow	sheep	rabbit	g-pig	chick	
Bauhinia Bonatiana	1	1	—	—	—	1	8	16	8	8	8	4	4	4						
— candicans	2	2	—	—	—	—	4	4	64	64	64	8	8	8	—	—	—	256	—	
— purpurea	2	2	—	—	—	—	4	4	64	64	64	4	4	4	—	—	256	—	—	99
— variegata	2	2	—	—	—	1	4	8												
Lens nigricans	2	2	8	8	8	—	4	4							—	—	64	64	—	
Vicia graminea	1	1	—	16	32	16	16	16												114, 94, 116, 84
— leganyana	4	2	16	16	16	—	4	8	32	32	32	32	32	32	—	—	64	32	—	
— picta	2	1	16	16	16	—	4	8	128	128	128	128	128	128						

3. ABSORPTION AND ELUTION EXPERIMENTS

On the basis of the foregoing it seems that some seed extracts, e.g. Vicia cracca extracts, contain agglutinins of two types with distinct properties. In Vicia cracca there is the type that agglutinates the cells of man, rabbit and guinea pig independently, as it seems, of blood groups and largely of the pH of the milieu. The other type is a strong anti-A agglutinin, which reacts at neutral and alkaline pH values only. Now the question arises whether these two types of agglutinin are separable from each other or whether they are components of one and the same molecule. With the purpose of finding a little more general reply to this question the author carried out absorption and elution tests with three extracts, which were prepared from the seeds of Vicia cracca, Bauhinia purpurea and Phaseolus vulgaris. The extract of Bauhinia purpurea agglutinated the cells of rabbit as well as, in serum milieu, human MN and N cells. Of the cells examined all but those of cow were agglutinated by the extract of Phaseolus vulgaris.

Crude extracts were used in the tests. They were carried out at room temperature using different kinds of washed red cells. In the absorption tests the ratios of the volume of the packed cells to that of the extract equalled 1: 4, 1: 2 and 1: 1. The elution tests were done according to the technique of Landsteiner and Miller (91).

When the Vicia cracca extract was absorbed with A_1 cells, the titre against these cells fell distinctly more than the titre against the cells of the blood group O, rabbit or guinea pig. If the extract was absorbed with one of these three kinds of cells, its titre against them all fell sharply while the titre of anti-A remained almost unchanged. Absorption of the Bauhinia purpurea extract with human M or N red cells or with the cells of rabbit did not appreciably influence the titres against these cells. The results obtained by means of the Phaseolus vulgaris extract were definite. No matter what kinds of cells were used, the titres against all of them fell, but the quality of the remaining agglutinins seemed to be the same in all cases.

The results from the elution experiments were more uniform. The eluates, as a rule, were almost identical, qualitatively, with the original crude extract.

Although the Bauhinia purpurea extract did not agglutinate human M cells, these cells when treated with the extract yielded an agglutinin, as an eluate, that reacted with the cells of rabbit and human N cells like the original extract. A similar eluate was obtained from human N corpuscles and the cells of rabbit.

DISCUSSION

A possible source of error in the experiments of this chapter (as well as in those of the preceding chapter) is faulty identification of plant species by the dispatching garden. In those cases, however, where a new blood group specific seed agglutinin was found by the present author, the garden concerned was requested to send another seed sample of the species in question. Such a control sample was generally received and always found to be identical with the original. Several samples of a species from different gardens were often available containing an identical agglutinin.

Extracts that prove blood group specific when tested in saline milieu usually appear as such also in kollidon milieu or when tested with papain treated cells as is shown in Table 7. Since the titres are generally higher when determined by either of these methods than they are in saline, it is possible that e.g. the anti-H extract of Laburnum alpinum agglutinates papainised A_1 cells. This does not mean, however, that the extract would agglutinate papainised cells unspecifically, as Dufner and Matthes (34) believe, for concentrated Laburnum alpinum agglutinins cause a clumping of A_1 cells in saline, too (101).

Investigators have been intrigued by the nature of plant agglutinins, and of those in particular which are blood group specific. The majority are of the opinion that they are hardly to be regarded as antibodies. The question that remains unsettled is whether the blood group specificity of certain plant agglutinins is absolute, i.e. whether they react with the same receptors as e.g. iso-anti-A or human immune anti-A.

The answer of Boyd and Shapleigh (25) to this question seems to be negative, that of Krüpe (84 p. 43) affirmative. Kabat (63), studying the inhibiting effect of both H substance and L-fucose on the anti-H agglutinins of eel serum and Lotus tetragonolobus

concludes that «the reactive site on the plant hemagglutinin is directed to a smaller unit on the blood group substance, perhaps an L-fucoside grouping, than is the reactive site on the eel anti-O(H)».

The present author supports the view of Boyd and Shapleigh who consider the blood group specificity of plant agglutinins to be accidental. On this ground one is tempted to develop Kabat's empirically grounded view still further. An assumption that suggests itself is that the reactive site of plant agglutinins in general is directed to a smaller unit than is the reactive site of animal agglutinins. It is more probable that chance produces a configuration complementary to a certain structure RTUT than that it does one complementary to another structure RTUTVXTYW in which the letters may be thought of as corresponding to certain groupings on the red cell surface. In the author's opinion this theory is supported by the fact that most plant agglutinins, whether blood group specific or no, are neutralised by certain simple sugars (Tables 9—11) whereas human agglutinins are not.

As will be seen from Table 6 there appears to be an almost continuous series of extracts from the most exclusively anti-A_1 specific Dolichos biflorus to the most exclusively anti-B specific Bandeiraea simplicifolia (Dolichos biflorus, Phaseolus lunatus, Vicia cracca, Crotalaria, Caragana frutex var. latifolia, Sophora japonica, Coronilla varia, and Bandeiraea simplicifolia). It seems improbable that a member of this series would react with the same receptor as human anti-A or anti-B.

For the above reasons the author thinks it improbable that the specific receptor of any of the plant agglutinins would be exactly similar to that of an isoagglutinin.

It seems that plant agglutinins do not behave exactly like animal agglutinins in absorption and elution tests. This is suggested by the poor absorbability of Bauhinia extract and to some extent by the contradictory results from the absorption and elution tests of Vicia cracca extract. This may also account for the somewhat contradictory reports on the separability of the anti-A and anti-B agglutinins of Sophora japonica (77, 108, 80, 143) and also the anti-A and anti-rabbit agglutinins of Vicia cracca (80). Krüpe (84) puts more confidence in the results from

the elution tests and accordingly concludes that the extracts of Sophora japonica and Vicia cracca contain but one agglutinin. Similar possibilities are implied by the present author's results from the extracts of Bauhinia purpurea and Phaseolus vulgaris, and somewhat similar observations have been made by earlier investigators (121, 50).

This investigation has perhaps thrown light on some useful principles, which it pays to observe if the sole purpose is to search for new blood group specific plant agglutinins. AB serum milieu is likely to be useful because there are blood group specific agglutinins that react in serum but not in saline milieu, and because the serum neutralises several unspecific agglutinins. In the family Leguminosae there are genera such as Acacia, Cassia and the whole tribe Trifolieae, in which agglutinins are not likely to be found.

Section two: INHIBITION OF AGGLUTINATION

SURVEY OF LITERATURE

1. INHIBITION BY NORMAL AND IMMUNE SERUM

It has been known for a long time that normal serum influences agglutination by plant extracts in two ways. The promoting influence of the serum was treated in the preceding chapters of this paper. The frequent opposite effect was also mentioned. Kraus (72) was the first to make a detailed study of the inhibiting property of the serum. He came to the conclusion that the agglutination of red cells and the precipitation in the serum was brought about by one and the same principle in a plant extract. After the plant agglutinin had precipitated with the serum proteins, the supernatant was no longer capable of agglutinating red cells. Kraus's theory was supported by the tests of Landsteiner and Stankovic (88), which showed that the agglutinins of ricin and abrin are bound by casein, fibrin, serum protein, and silk. Subsequent investigations, however, have not confirmed Kraus's theory (120) (See page 17). Instead, many investigators have noticed the inhibiting effect of normal serum on several plant agglutinins (50, 103, 31). Craeger and Gifford (31) have studied this problem more closely and they conclude that of the

serum proteins the gamma globulin is primarily instrumental in the inhibition of phytagglutination.

Anti-plant protein sera produced by immunisation are capable of inhibiting an agglutination caused by the extracts of the plant species whose protein was used in the immunisation. Sometimes they inhibit an agglutination caused by closely related species, too.

2. INHIBITION BY BLOOD GROUP SUBSTANCES

Since Yamakami (150) 1926 observed that human seminal fluid and saliva were capable of specific inhibition of isoagglutination, various mucins have been found to possess blood group specific activity. In fact, ability of substances similar to those mentioned above to neutralise the action of crotin, abrin and ricin on red cells was known much earlier (59, 97, 90, 119). These substances were derived from sources very similar to those from which the present blood group substances are obtained.

The investigators mentioned above did not succeed in identifying the substance that inhibited the action of crotin, abrin and ricin. The matter was soon forgotten, and it was not until after the blood group specific agglutinins had been found that fresh interest was shown in the inhibition of phytagglutination by mucins. Krüpe (75) and Koulumies (71) were the first to show that soluble blood group substances neutralise the anti-H agglutinin of Laburnum alpinum, Lotus tetragonolobus and Cytisus sessilifolius. Morgan and Watkins (108) have carried out extensive studies, in which they have used blood group substances from different sources. Agglutination by all the blood group specific plant agglutinins examined was inhibited by the corresponding blood group substances. The agglutinins of Sophora japonica that are known to agglutinate A and B cells strongly were inhibited by both A and B substances. Quantitative differences occurred all the same, thus the anti-H agglutinin of Lotus tetragonolobus was neutralised by the H substance much more poorly than was the anti-H of Cytisus sessilifolius. Krüpe (84) found that some unspecific plant agglutinins, too, were neutralised by blood group substances. Krüpe (82) found that,

like the incomplete isoagglutinins, the incomplete plant agglutinins as well are far less easily neutralised by soluble blood group substances than are the complete plant agglutinins.

Tétry et coll. (141, 142) reported that by absorption with a suitable saliva they had managed to prepare blood group specific reagents from fungous extracts that had agglutinated almost unspecifically to start with. In one case the saliva even of a nonsecretor was usable for this purpose.

Substances capable of neutralising anti-A, anti-B and anti-H of different origins have been discovered by Springer (136, 137, 138) in different parts of Taxus cuspidata and some other plants. Springer (138) and Krüpe (84) have noticed that some plant agglutinins, too, are neutralised by the extracts of Taxus cuspidata.

3. INHIBITION BY SIMPLE SUGARS

The classic experiments of Landsteiner (92) proved that very simple substances are capable of acting as the determining groups of antigens. It has also been known for a long time that a substance of low molecular weight whose molecule contains the determining group of an antigen may neutralise an immune serum produced by means of the antigen (3).

In 1952 Watkins and Morgan (145) showed first that simple sugars are capable of neutralising normal agglutinins. The anti-H agglutinin that occurs in the serum of eel was neutralised by L-fucose and some stucturally related compounds. Actually as early as 1936 it was noticed by Sumner and Howell (140) that agglutination by concanavalin A, a seed globulin, was inhibited by cane sugar. The causes of the inhibition were not studied, however.

The observation of Watkins and Morgan was of great importance, because an obvious explanation of the phenomenon was that the sugars whose structure most closely resembles the specific groups of a red cell receptor attach themselves to the active sites of the agglutinin thus blocking them. Subsequent investigations have shown, however, that simple sugars inhibit only few animal agglutinins with the exception of these heterologous anti-H agglutinins.

Thanks to the investigations of Morgan and Watkins (108) and Krüpe (83, 84) information is available at present about the neutralisation of many blood group specific plant agglutinins by different kinds of sugars, amino acids and polysaccharides including those which are known to be integral parts of blood group substances. All amino acids examined have been found to be inactive, which is also true of most polysaccharides. Several simple sugars, on the contrary, have turned out to be capable of neutralising different plant agglutinins.

Agglutination-inhibition experiments have shown that anti-H reagents from different sources are of two kinds. Morgan and Watkins (108) found that the anti-H agglutinin of Lotus tetragonolobus was inhibited by small amounts of L-fucose, unlike the anti-H agglutinins of Cytisus sessilifolius and Laburnum alpinum. Krüpe (83) found later that salicin neutralised many agglutinins of the latter group but not those of eel serum and Lotus tetragonolobus.

The plant agglutinins showing anti-A specificity were found to be neutralised by N-acetylgalactosamine though this substance was needed in a much greater quantity than L-fucose to inhibit agglutination by Lotus tetragonolobus extract.

The agglutinins of Sophora japonica and Coronilla varia, too, that reacted with both A and B cells were inhibited by N-acetylgalactosamine. This fact is of some interest, because N-acetylgalactosamine is present in the hydrolysates of blood group A substance, while much smaller amounts can be split from O substance and only traces from B substance (63). These two plant agglutinins were also inhibited by some other sugars, e.g. D-galactose (known to be an integral part of human blood group substances) and lactose. None of the plant agglutinins showing anti-A or anti-B specificity was affected by L-fucose, and so Kabat (63) believed that this sugar is hardly a major factor in A and B specificity. Although N-acetylglucosamine is an integral part of human blood group substances, none of the blood group specific plant agglutinins was neutralised by it.

The remarkable affinity of sugars for the active groups of plant agglutinins is illustrated by the fact that if a proper kind of sugar is added to a mixture of plant extract and red cells

showing visible agglutinates, the cell aggregates disintegrate almost at once (84).

Also such plant agglutinins as show no human blood group specificity are neutralised by various sugars (83, 84). The amounts of sugar necessary are, as a rule, greater than those of L-fucose, N-acetylgalactosamine and D-galactose needed to inhibit agglutination by blood group specific plant agglutinins.

To the best of the author's knowledge no studies have been published concerning the inhibition by simple sugars of the precipitation of blood group substances with seed extracts.

OWN INVESTIGATIONS

I. MATERIAL AND METHODS

Experiments on the inhibition by normal human serum of seed agglutinins were carried out automatically in the tests of Chapter II. Discussion of the results is continued in this chapter. Actual inhibition tests were all carried out as follows.

A solution of a given plant agglutinin and the substance to be examined were mixed and the red cell suspension was added to the mixture. If an agglutination did not follow, the substance could be said to be capable of inhibiting an agglutination by the seed extract as the conditions were under control.

The tests were so arranged that the amount of agglutinin was constant, with the final agglutination mixture containing approximately four times the minimum amount of agglutinins needed for an agglutination to take place. The total volume of the reaction mixture and the red cell concentration were also kept constant. The concentration of the substance to be studied decreased in geometric progression, the ratio of whose consecutive terms was 1/2. In this way inhibition titres were obtained for the different substances.

Both blood group specific and unspecific extracts were used as the material.

Specimens of the latter group were chosen so that as many genera and tribes as possible might be represented.

Human red cells of the various blood groups were used in the main, but also the red cells of some animals.

The tests were carried out at room temperature. Of the plant extract diluted in 0.9 % NaCl solution, 0.1 ccm followed by 0.1 ccm of the substance to be studied in 0.9 % NaCl solution was put in test tubes. The substances were mixed and 0.1 ccm of 3 % red cell suspension in 0.9 % NaCl solution was added after half an hour. When it was desired to examine the inhibition of the indirect agglutination, the plant extract was diluted in AB serum, in which also the red cells were suspended. Accordingly, the serum concentra-

tion of the final solution was approximately 2/3. In some cases also papain treated cells were used.

Two groups of substances were studied with regard to their capability of inhibiting phytagglutination:

The blood group substances:

— The salivas of secretors belonging to the blood groups A_1, B and O; and of a nonsecretor belonging to the blood group O. A dilution 1: 4 was used as a stock solution.

— A solution of blood group substances supplied by the firm of Sharp & Dohme, Philadelphia, Pa. U.S.A. It was derived from pig and horse gastric mucosa and contained A, B and H substances. Further on in this paper it will be referred to as «blood group substance».

Following simple sugars and related substances:

N-acetyl-D-galactosamine, galactosamine, D-galactose, lactose, melibiose, raffinose, L-galactose, N-acetylglucosamine, D-glucose, maltose, sucrose, salicin, D-mannose, D-fructose, L-sorbose, L-rhamnose, L-fucose, D-digitoxose, L-arabinose, D-arabinose, D-ribose, xylose, sorbitol, mannitol, dulcitol, and inositol.

N-acetylglucosamine was received from Heyl & Co., Berlin, Germany; N-acetylgalactosamine and galactosamine from Serva Entwicklungslabor., Heidelberg, Germany. The rest of the substances were obtained by the suppliers without difficulty.

A 2 % stock solution in 0.9 % NaCl was prepared of these substances, each, with the exception of N-acetyl-D-galactosamine, the initial concentration of which was 0.2 %.

2. INHIBITION BY NORMAL SERUM

As will be seen from the principal Table 5 in Chapter II, the agglutination of human red cells by many seed extracts is inhibited by human serum. The titres of the Vicia extracts for instance may be 1/32 in saline milieu, whereas in 67 % serum agglutination will not be brought about even by the undiluted extract tested with the same cells. The author did not encounter marked differences in the agglutination-inhibiting power between the sera of various adults.

The serum does not seem to inhibit the blood group specific agglutinins. The extract of Vicia dumetorum, for example, behaves in an interesting manner. In saline milieu it agglutinates all human red cells the titre being 1/8. As the serum content of the reaction milieu is increased, the agglutination of B and O

cells weakens, but that of A cells becomes stronger until, in the 67 % serum, A_1 cells are agglutinated at a dilution of 1/64 but B and O cells not at all.

3. INHIBITION BY BLOOD GROUP SUBSTANCES AND SIMPLE SUGARS

The results from the inhibition experiments are presented in Tables 9—11. Of the examined carbohydrates all four polyhydric alcohols, sorbitol, mannitol, dulcitol, and inositol, as well as the pentoses D-ribose and xylose were totally incapable of inhibiting an agglutination in the concentration 1:50. These findings are not included in the tables.

None of the anti-N specific agglutinins studied (Bauhinia candicans, Bauhinia purpurea, Vicia leganyana) was inhibited by any of the substances examined. The same is true of the examined hemolysing extracts Albizzia stipulata, Medicago rigidula and Dolichos falcata. These findings are not included in the tables.

The results from the tests carried out by means of anti-A, anti-B and anti-A+B specific agglutinins are given in Table 9. With one exception the agglutination of A_1 cells by anti-A specific plant agglutinins studied was inhibited by the commercial blood group substance and the saliva of an A_1 secretor.

None of them were neutralised by other salivas. Most of these agglutinins were inhibited by N-acetyl-D-galactosamine. D-galactose and oligosaccharides containing D-galactose seem to inhibit some of the anti-A specific plant agglutinins slightly. (All the studied oligosaccharides containing D-galactose were galactosides.) The anti-A agglutinin of Vicia dumetorum, which only shows this specificity in serum milieu, was not inhibited by soluble blood group substances or sugars.

The agglutination of B cells by the anti-B specific extract made from Bandeiraea simplicifolia seeds harvested a year before was inhibited by B substance, N-acetyl-D-galactosamine, galactosamine, D-galactose, and oligosaccharides containing D-galactose (galactosides), moreover, by L-rhamnose and L-arabinose. Almost the same is true of the agglutination of cow and rabbit erythrocytes by the extract.

Plant agglutinins of the type anti-A+B constitute a group that is diverse botanically and also serologically, to some degree,

TABLE 9. The inhibition of the agglutination of human A_1 and B red cells by anti-A, anti-A+B, and anti-B specific plant agglutinins, by blood group substances as well as by simple sugars and related compounds.

	Initial concentr.	Lathyrus pratensis	Vicia cracca		Vicia dumetorum	Vicia varia		Vicia villosa	Amphicarpaea Edgeworthii	Dolichos biflorus	Phaseolus lunatus	(isoaggl. anti-A)
		A_1 ser	A_1 NaCl	A_1 ser	A_1 ser	A_1 NaCl	A_1 ser	A_1 NaCl	A_1 NaCl	A_1 NaCl	A_1 NaCl	A_1 NaCl
blood group subst.	1:1	256	128	128		256	128	64	256	64	32	512
saliva of a A_1	1:4	8	64	64		64	64	8	16	128	32	64
secretor, B	1:4											
blood group O	1:4											
saliva of nonsecretor	1:4											
N-acetylgalactosam.	1:500		4000	2000		500	500	500	1000	500?	500?	
galactosamine	1:50											
D-galactose	1:50	100							50			
lactose	1:50	50							100			
melibiose	1:50	200	100	50					400			
raffinose	1:50	400	50	50					200			
L-galactose	1:50											
N-acetylglucosamine	1:50											
D-glucose	1:50											
maltose	1:50											
sucrose	1:50											
salicin	1:50											
D-mannose	1:50											
D-fructose	1:50											
L-sorbose	1:50											
L-rhamnose	1:50											
L-fucose	1:50											
D-digitoxose	1:50											
L-arabinose	1:50											
D-arabinose	1:50											

The denominators of the final concentrations of the substances studied, capable of inhibiting the agglutination, are given in the table. Blank space indicates failure of inhibition by the initial concentration given in the first column. Test cells and reaction milieu are described below the name of the plant species. (NaCl = saline milieu, ser = AB serum milieu, pap = papain treated cells in saline)

TABLE 9 (cont'd)

TABLE 9 (cont'd)

	Initial concentr.	Crotalaria compista	Crotalaria striata	Caragana frutex var. latifolia		Dolichos lab-lab		Vigna catjang		Sophora japonica [1]	Sophora japonica		Coronilla varia	Bandeiraea simplicifolia [2]		Bandeiraea simplicifolia [3]	(isoagg. anti-B)
		A_1 NaCl	A_1 NaCl	A_1 ser	B ser	A_1 ser	B ser	A_1 ser	B ser	B NaCl	A_1 ser	B ser	B ser	A_1 NaCl	B NaCl	B NaCl	B NaCl
blood group subst.	1:1		8	64	64								128	8	8	8	256
saliva of a A_1	1:4	8	16	16	8	32		32		4	16	4	128	8			
secretor, B	1:4			8	8		32		32	32	32	16	128	8	16	32	64
blood group O	1:4																
saliva of nonsecretor	1:4																
N-acetylgalactosam.	1:500									1000	500	1000	1000	2000	4000	4000	
galactosamine	1:50									100	50	50	100	400	400	400	
D-galactose	1:50	100		100	100	200	400	400	400	400	400	200	200	1600	3200	1600	
lactose	1:50			100	100	50	100	100	100	400	400	400	100	200	200	200	
melibiose	1:50		100	400	400	50	200	50	400	200	100	100	800	1600	1600	1600	
raffinose	1:50		100	400	400		50			100	50	50	200	1600	1600	1600	
L-galactose	1:50																
N-acetylglucosamine	1:50																
D-glucose	1:50													400			
maltose	1:50																
sucrose	1:50													800			
salicin	1:50																
D-mannose	1:50																
D-fructose	1:50																
L-sorbose	1:50																
L-rhamnose	1:50													200	200	200	
L-fucose	1:50																
D-digitoxose	1:50									50		50	100				
L-arabinose	1:50					200	200	200	200	200	200	200	200	400	400	400	
D-arabinose	1:50																

[1] aged extract [2] recently harvested seeds [3] one year old seeds

TABLE 10. The inhibition of the agglutination of human O red cells by anti-H specific plant agglutinins, by blood group substances as well as by simple sugars and related compounds. — (Explanations are in Table 9 p. 112).

		Initial concentr.	Virgilia lutea O ser	Cytisus argenteus O NaCl	Cytisus benehoa-vensis O NaCl	Cytisus lusitanicus O NaCl	Cytisus nigricans O NaCl	Cytisus purpureus O NaCl	Cytisus sessilifolius O NaCl	Genista sagittalis O NaCl	Genista sphaerocarpa O NaCl	Genista umbellata O NaCl	Laburnum alpinum O NaCl	Petteria ramentacea O pap.	Ulex europaeus O NaCl	Ulex Gallii O NaCl	Ulex nanus O NaCl	Lotus siliquosus O NaCl	Lotus tetragono-lobus O NaCl
blood group subst.		1:1	512	512	256	256	256	512	512	512	256	256	64	512	1024	1024	256	8	4
saliva of a	A_1	1:4	64	32	16	32	16	32	32	32	16	16	16	4	128	256	32	8	8
secretor,	B	1:4	16	16	16	16	16	16	32	16	16	16	16	4	32	128	32	8	8
blood group	O	1:4	64	64	16	32	16	32	32	64	16	32	16	4	128	128	64	8	8
saliva of nonsecretor		1:4																	
N-acetylgalactosam.		1:500																	
galactosamine		1:50										50			200		400		100
D-galactose		1:50	200																
lactose		1:50	200	50	200	400	100	200	400	400	400	200	400	100					200
melibiose		1:50																	
raffinose		1:50																	
L-galactose		1:50																400	1600
N-acetylglucosamine		1:50																	400
D-glucose		1:50																	50
maltose		1:50																	
sucrose		1:50																	
salicin		1:50		200	200	400	200	50	200	100	100	100	100	100	200	100	100		50
D-mannose		1:50																	50
D-fructose		1:50																	
L-sorbose		1:50																	
L-rhamnose		1:50																	
L-fucose		1:50													3200		3200	6400	12800
D-digitoxose		1:50																50	400
L-arabinose		1:50																	
D-arabinose		1:50																100	

for some of them cause a stronger agglutination of A cells, others of B cells. In agglutination-inhibition tests the reactions of the group were more homogeneous. The agglutination of A and B cells was inhibited by D-galactose and/or compounds containing this sugar. In many cases it was inhibited by L-arabinose, too.

The inhibition of these agglutinins by blood group substances is of interest. The agglutination of A_1 and B cells is frequently inhibited by both A and B substances. This does not seem to apply to the agglutinins of Vigna catjang and Dolichos lablab. The agglutination of A_1 cells is inhibited by A substance, that of B cells by B substance, only.

The results from the inhibition experiments with anti-H specific plant agglutinins are given in Table 10. They indicate that the anti-H agglutinins of the genus Virgilia and the tribe Genisteae (Cytisus, Genista, Laburnum, Petteria, Ulex) are inhibited by H substance and by salicin. The agglutinins of the genus Ulex are, moreover, neutralised by L-fucose. Most of the other anti-H agglutinins of the tribe Genisteae are inhibited by lactose. The anti-H agglutinins of Lotus tetragonolobus and Lotus siliquosus, on the other hand, are less readily inhibited by H substance but strongly by L-fucose.

Table 11 shows the results from inhibition tests carried out by such extracts as do not agglutinate human red cells selectively, as well as from tests that were done with blood group specific extracts using animal red cells or a method by which specificity is not manifested. Thus the table shows the results from the inhibition tests in which anti-A specific Vicia cracca extract was used against the cells of rabbit as well as from tests in which anti-A specific Vicia dumetorum extract was used against A_1 cells in saline milieu. This was done because the anti-A specificity of the extract does not become manifest in saline milieu. The plant species are grouped according to the taxonomic system and in such a way that the results from a given extract using different kinds of red cells are presented in succession. The table is lengthy and shows that many «unspecific» plant agglutinins are inhibited by the examined substances. The results will be treated in the discussion of this chapter.

TABLE 11. The inhibition of some plant agglutinins by blood group substances as well as by simple sugars and related compounds.
(Explanations are in Table 9 p. 112. The order of the plant species is that of Table 5)

	Initial concentr.	Parkia filicoidea		Bandeiraea simplicifolia		Bauhinia variegata v. candida	Bauhinia sp?	Cladrastis amurensis			Sophora japonica	Virgilia divaricata		Crotalaria juncea		Crotalaria madurensis
		rabbit NaCl	g-pig NaCl	cow NaCl	rabbit NaCl	rabbit NaCl	rabbit NaCl	O NaCl	rabbit NaCl	g-pig NaCl	rabbit NaCl	cow NaCl	sheep NaCl	A_1 NaCl	rabbit NaCl	rabbit NaCl
blood group subst.	1:1			16										64	8	4
saliva of a A_1	1:4	4	4			32	16		32	32		16	8	128	64	8
secretor, B	1:4			16	8	8	4		32	32	8	128	64	64	64	8
blood group O	1:4	4	4			16	8		32	32		32	32	32	32	8
saliva of nonsecretor	1:4	16	16			16	8		32	32		16	16	32	32	8
N-acetylgalactosam.	1:500			4000		500	500				500					
galactosamine	1:50			400												
D-galactose	1:50			6400	1600	1600	1600				400			400	400	1600
lactose	1:50			50		1600	1600				400			1600	1600	800
melibiose	1:50			6400	800	800	800				200			400	400	800
raffinose	1:50			6400	400	400	200				50			50	50	100
L-galactose	1:50															
N-acetylglucosamine	1:50	100	100													
D-glucose	1:50	100	100													
maltose	1:50	400	400													
sucrose	1:50															
salicin	1:50					100	100									
D-mannose	1:50	800	800													
D-fructose	1:50															
L-sorbose	1:50															
L-rhamnose	1:50					200	200									
L-fucose	1:50															
D-digitoxose	1:50															
L-arabinose	1:50			200		200	200				200			100	100	
D-arabinose	1:50															

L-arabinose	1:50			200		200	200				200			100	100		
D-arabinose	1:50																

TABLE II (cont'd)

		Initial concentr.	Crotalaria verrucosa		Cytisus austriacus	Cytisus capitatus	Cytisus proliferus		Genista aethnensis			Lotononis cytisoides		Caragana arborescens		Caragana decorticans	
			A_1 NaCl	rabbit NaCl	rabbit NaCl	rabbit NaCl	O NaCl	rabbit NaCl	O NaCl	rabbit NaCl	g-pig NaCl	O NaCl	rabbit NaCl	A_1 NaCl	rabbit NaCl	A_1 NaCl	rabbit NaCl
blood group subst.		1:1	64	64	128	64	1024	256						512		16	
saliva of a	A_1	1:4	32	32	16	4	64	64	256	128	64	256	256	32	8	16	8
secretor,	B	1:4	16	16	16	8	32	64	64	64	64	256	256	16	4	16	4
blood group	O	1:4	16	16	16	4	128	64	64	64	64	256	256	64	4	16	4
saliva of nonsecretor		1:4	16	16		4			64	64	64	256	256	16	4	8	4
N-acetylgalactosam.		1:500												500	500	500	500
galactosamine		1:50														50	
D-galactose		1:50	800	800	800	800	800	1600						400		400	50
lactose		1:50	1600	800	3200	6400	800	1600						800		400	100
melibiose		1:50	800	800	1600	3200		800						400	50	200	50
raffinose		1:50	200	100	400	3200		400							50	50	50
L-galactose		1:50															
N-acetylglucosamine		1:50															
D-glucose		1:50															
maltose		1:50															
sucrose		1:50															
salicin		1:50						50									
D-mannose		1:50															
D-fructose		1:50															
L-sorbose		1:50															
L-rhamnose		1:50															
L-fucose		1:50															
D-digitoxose		1:50															
L-arabinose		1:50												50	50	50	
D-arabinose		1:50															

Continued

TABLE II (cont'd)

	Initial concentr.	Caragana decorticans	Caragana frutex		Caragana frutex v. latifolia	Robinia holdtii			Wistaria chinensis			Coronilla varia	Lathyrus latifolius			Lens culinaris	
		chick NaCl	A_1 NaCl	sheep NaCl	sheep NaCl	O NaCl	sheep NaCl	g-pig NaCl	O NaCl	rabbit NaCl	chick NaCl	rabbit NaCl	O NaCl	rabbit NaCl	g-pig NaCl	O NaCl	rabbit NaCl
blood group subst.	1:1								4	2		128					
saliva of a A_1	1:4	•		4	8							128	4			4	
secretor, B	1:4			4	8							128	8	4		4	4
blood group O	1:4			4	8								8			4	
saliva of nonsecretor	1:4			4	8								16	8	4	8	4
N-acetylgalactosam.	1:500											500					
galactosamine	1:50																
D-galactose	1:50								200	200		200					
lactose	1:50								800	800		50					
melibiose	1:50								400	400		200					
raffinose	1:50								200	200		100					
L-galactose	1:50																
N-acetylglucosamine	1:50												400	400	200	400	200
D-glucose	1:50												400	400	400	400	400
maltose	1:50												400	400	200	400	400
sucrose	1:50												200	100	100	200	100
salicin	1:50												50	100		50	100
D-mannose	1:50												800	400	400	800	400
D-fructose	1:50												100	100	50	100	
L-sorbose	1:50												100				
L-rhamnose	1:50																
L-fucose	1:50																
D-digitoxose	1:50											100					
L-arabinose	1:50											200					
D-arabinose	1:50																

TABLE 11 (cont'd)

		Initial concentr.	Lens culinaris	Pisum sativum			Vicia cracca (anti-A)			Vicia dumetorum				Vicia nana			Atylosia barbata	
			g-pig NaCl	O NaCl	rabbit NaCl	g-pig NaCl	O NaCl	rabbit NaCl	g-pig NaCl	A_1 NaCl	O NaCl	rabbit NaCl	g-pig NaCl	O NaCl	rabbit NaCl	g-pig NaCl	O NaCl	rabbit NaCl
blood group subst.		1:1																
saliva of a	A_1	1:4		4			4			4	4			4				4
secretor,	B	1:4	4	4	4	4	8		4	4	4	4	4	4	4	4		4
blood group	O	1:4		4			4			4	4			4				4
saliva of nonsecretor		1:4	4	8	4	4	8	8	16	4	8	8	8	4	4	4		4
N-acetylgalactosam.		1:500																
galactosamine		1:50																
D-galactose		1:50																
lactose		1:50																
melibiose		1:50																
raffinose		1:50																
L-galactose		1:50																
N-acetylglucosamine		1:50	400	400	400	400	200	100	100	100	100	100	200	100	100	200		
D-glucose		1:50	200	400	400	400	400	400	400	400	400	400	400	400	400	400		
maltose		1:50	200	400	400	400	200	200	400	400	400	400	400	800	400	400		
sucrose		1:50	200	400	400	400	100	100	200	200	100	100	100	200	200	200		
salicin		1:50	50	200	200	200	50	50	50	50	50	50	50	50	100	100		
D-mannose		1:50	400	800	800	800	400	800	400	400	400	400	800	800	800	400		
D-fructose		1:50	50	200	200	200	100	200	200	100	100	100	100	100	200	100		
L-sorbose		1:50	50				100		50	50	50	50	50					
L-rhamnose		1:50																
L-fucose		1:50																
D-digitoxose		1:50																
L-arabinose		1:50																
D-arabinose		1:50																

Continued

TABLE II (cont'd)

		Initial concentr.	Atylosia barbata	Canavalia ensiformis				Dolichos lab-lab (non-specific)				Erythrina indica		Erythrina vespertilio	Glycine soja			Phaseolus coccineus
			g-pig NaCl	O NaCl	sheep NaCl	rabbit NaCl	g-pig NaCl	A_1 NaCl	A_1 ser	rabbit NaCl	g-pig NaCl	O NaCl	chick NaCl	O NaCl	A_1 pap	O pap	rabbit NaCl	O NaCl
blood group subst.		1:1										8	1	4	64	64	32	
saliva of a	A_1	1:4	4			16						4						4
secretor,	B	1:4	4			8						4		4				4
blood group	O	1:4	4			4												4
saliva of nonsecretor		1:4	4			4												4
N-acetylgalactosam.		1:500															1000	
galactosamine		1:50																
D-galactose		1:50										1600	1600	1600	200	200	800	
lactose		1:50										3200	3200	3200	100	100	400	
melibiose		1:50										1600	3200	1600	400	400	800	
raffinose		1:50										800	400	400	100	100	400	
L-galactose		1:50										200	200	200				
N-acetylglucosamine		1:50																
D-glucose		1:50																
maltose		1:50																
sucrose		1:50																
salicin		1:50																
D-mannose		1:50																
D-fructose		1:50																
L-sorbose		1i50																
L-rhamnose		1:50										50	50	50	50			
L-fucose		1:50																
D-digitoxose		1:50																
L-arabinose		1:50										100	100	100	200	200	200	
D-arabinose		1:50																

DISCUSSION

When the results from the tests of this chapter are studied, it will be noticed that the initial and final concentrations of the substances inhibiting agglutination are not very wide apart. In the case of the sugars the highest concentration studied was generally but 2—16 times the final inhibiting concentration. Almost the same is true of the inhibiting power of the salivas. It is possible, admittedly, that some sugars which now proved inactive would have inhibited the agglutination by some of the extracts if the initial concentration had been higher. As several of the sugars, however, were rather expensive and because undiluted saliva would have changed the viscosity of the solutions, the arrangement described above was decided upon.

The results are in agreement, by and large, with the results obtained by other investigators (108, 84). The inhibition titres of N-acetyl-D-galactosamine are lower, on an average, in the present investigation than they are in other authors.

The present author's results show that also many incomplete agglutinins are inhibited by blood group substances and simple sugars. The anti-A agglutinin of Vicia dumetorum, however, only demonstrable in serum milieu, was not inhibited by any of the studied substances.

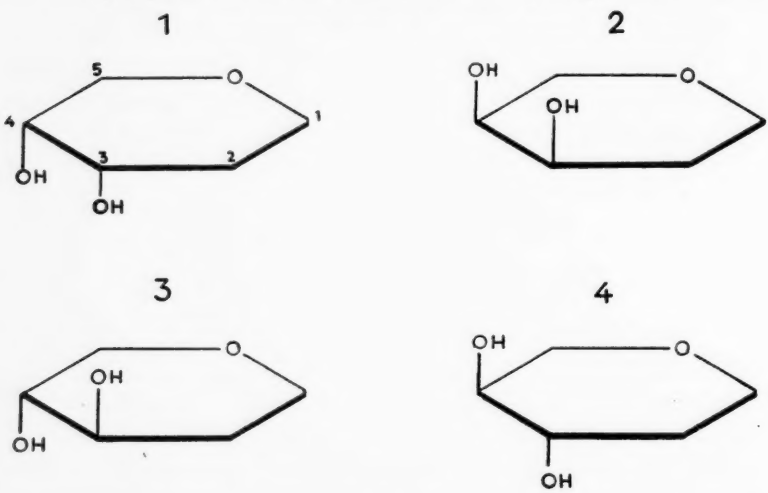
The present author's results indicate that human saliva contains a substance that is capable of inhibiting plant agglutinins and which is secreted by both secretors and nonsecretors. This substance apparently does not inhibit blood group specific plant agglutinins while it inhibits many other plant agglutinins. By studying the ability of 50 samples of human saliva to inhibit an agglutination of human O cells caused by unspecific Lathyrus latifolius and Pisum Jomardi seed extracts quantitative differences were found between the samples. They did not relate clearly to the donor's secretor type (101).

The identity of the substance in question with Le^a substance is not excluded but seems improbable. The commercial mixture of the blood group substances A, B and H derived from hog and horse gastric mucosa does not seem to contain detectable amounts of this substance because many seed extracts were inhibited by saliva but not by the mixture.

Tétry et coll. (142) report that the extract from the fungus Xylaria polymorpha, an originally unspecific agglutinant of human red cells, agglutinated only O and A_2 cells, and B cells weakly after it had been absorbed with any saliva. The saliva of a nonsecretor, however, was less effective for this purpose than that of a secretor. This observation has not been confirmed as far as the present author knows. The seed extract of Vicia nana whose titre against human erythrocytes fell noticeably after an absorption with both secretor and nonsecretor saliva, agglutinated the cells unspecifically even after the absorption (101).

Both Morgan and Watkins (108) as well as Krüpe (84) suggest that the nature of the substituents in the carbon atoms 3 and 4 is of importance to the capability of sugars of inhibiting agglutinins. Changes in these substituents resulted in definitely changed activity of the sugar, which did not necessarily follow changes in the substituents of the carbon atoms 2 and 6.

Fig. 3. — Showing the four patterns possible in the placement of hydroxyl groups in the carbon atoms 3 and 4 of (aldo)pyranoses



Aldopyranoses are divisible into 4 groups according as the hydrogen atoms and hydroxyl groups are arranged in the carbon atoms 3 and 4 (Fig. 3). Krüpe (84) has noticed that the more strongly inhibiting sugars all belong to the groups

1 and 2 of the figure. He says that many sugars of the group 1 (L-galactose, L-fucose, D-digitoxose and D-arabinose) inhibit anti-H extracts and those of the group 2, such as D-galactose and L-arabinose, inhibit anti-B extracts (anti-A+B extracts from Sophora japonica and Coronilla varia).

The results from this investigation show that the sugars also of the group 3 of Fig. 3 (D-glucose and D-mannose as well as D-fructose which, although a ketose, has a similar pyranose ring) strongly inhibit plant agglutinins of types, it is true, different from those mentioned in the preceding paragraph. Indeed, most of the «unspecific» agglutinins of Table 11 seem to belong to one of three groups: those which are inhibited by the sugars of the 2nd group or 3rd group of Fig. 3, or those which are inhibited by none of the studied sugars.

The author's results obtained from thus far unexamined plant species confirm the observation of Morgan and Watkins (108) as well as of Krüpe (83) that anti-A+B specific plant agglutinins are inhibited by N-acetyl-D-galactosamine, D-galactose (and galactosides). Many of these agglutinins are inhibited by the structurally related L-arabinose, too. The most purely anti-B specific Bandeiraea simplicifolia, too, belongs to the same category. It has been found that also the precipitation of animal anti-B by B substance is inhibited by N-acetyl-D-galactosamine, D-galactose and some galactosides (61, 62).

In the preceding chapter the author came to the conclusion that in many, if not all, plant agglutinins, the active groups, which react with different kinds of red cells, are all in the same molecule. The question that still remains is whether these active groups are identical or not. Krüpe (84) seems to adopt the former view. On page 53 of his monograph he concludes that both human A cells and the cells of rabbit, guinea pig and hog possess a common partial antigen reactive with the agglutinin of Vicia cracca.

Agglutination-inhibition experiments on an extract by means of different kinds of cells may provide an answer to this question. It will be seen from Table 11 e.g. that the agglutination of the red cells of rabbit by the seed extracts of Sophora japonica and Coronilla varia is inhibited by the same compounds as the agglutination of the A_1 and B cells caused by the same extracts.

It would be possible to quote still other examples of this kind. Krüpe's theory seems to apply to these cases.

It seems, however, that Krüpe's theory does not hold true of absolutely all cases. In Chapter I of this investigation it was already observed that in an acid solution the anti-A agglutinin of Vicia cracca reacts very poorly, while the anti-rabbit and anti-guinea pig agglutinins of the same extract react well. Thus one is inclined to surmise that there may be two kinds of active groupings in the agglutinin molecule of Vicia cracca: anti-A groupings and anti-rabbit+guinea pig (+man) groupings. This view is supported by the results from the agglutination-inhibition tests. Agglutination of A_1 cells by Vicia cracca extract is inhibited by A substance and N-acetyl-D-galactosamine, while agglutination by the same extract of human O, rabbit and guinea pig red cells is inhibited by all types of human saliva, including the nonsecretor type, and by the sugars of the group 3 of Fig. 3. The anti-man+rabbit+guinea pig agglutinin accordingly seems to be identical with the common unspecific Vicia agglutinins.

The results given in Tables 5 and 11 allow one to conclude that the red cells of rabbit contain at least two different such receptors as react with different plant agglutinins. There are seed extracts that agglutinate the red cells of rabbit but not those of guinea pig, and the agglutinin is inhibited by D-galactose and D-galactosides. This group seems to comprise the anti-rabbit agglutinins e.g. of the genera Bandeiraea, Sophora, Crotalaria, Cytisus, Caragana, and Coronilla. Most of these extracts contain also anti-A+B or anti-B agglutinins. There are such extracts, too, as agglutinate besides the red cells of rabbit, also the cells of man and guinea pig; and the agglutination is inhibited by the sugars of the 3rd group of Fig. 3. The agglutinins of the genera Lathyrus, Lens, Pisum, Vicia, and perhaps Parkia belong to this group.

The observation of the cognate properties of the agglutinogens of human B red cells and the red cells of rabbit is in agreement with the corresponding findings made in 1933 by Friedenreich and With (52). It is interesting that the most purely anti-B specific plant extract from the seeds of Bandeiraea simplicifolia agglutinates, in addition to the cells of rabbit, also those of cow, and

that the agglutination of all three kinds of cells is inhibited by B substance but not by A or H substance. Both anti-B and anti-cow agglutinins are probably scarce in the vegetable kingdom.

Endeavours to improve the specificity of plant extracts by means of red cell absorption have seldom yielded satisfactory results. This stands to reason if the extracts contain agglutinin molecules of only one kind. The findings of this chapter maybe offer a different means of increasing the specificity. If an agglutinin molecule contains prosthetic groups of more than one kind, it may be possible to saturate some of them with soluble substances so that receptors of only one kind remain active.

Indeed, this has been accomplished (101). In saline milieu an extract made from newly harvested seeds of Bandeiraea simplicifolia agglutinated, besides both human B red cells and the cells of cow and rabbit, human A_1 cells, too. Agglutination tests that were done using a cell suspension that contained 2 % sucrose and 0.9 % NaCl showed agglutination of B cells by the same extract at the final dilution of 1/128. A_1 cells were not agglutinated by the extract at all.

SUMMARY

The hemagglutinins of 1408 seed samples representing 743 plant species and 165 genera, all of the family Leguminosae, were studied in the present investigation.

At first, the effects of physical and chemical factors on phytagglutination were examined by means of a few seed samples. It was found that changes in temperature between +4° and +37°C had no appreciable effect, which was also true of changes in hydrogen ion concentration between pH 4.5 and pH 11.0, as a rule. Agglutination by the seed extracts often did not take place in a milieu poor in electrolytes and was weak at best. The agglutination titre of all extracts rose when the NaCl concentration was raised until molarity 0.15 (0.9 %) was reached. A further rise in the salt concentration up to molarity 0.8 (4.5 %) was accompanied by a fall in the agglutination titre; still further rise in the concentration entailed a new rise also in the titre. The causes of this phenomenon are discussed.

Different methods for detecting incomplete plant agglutinins were compared by means of two seed extracts. Agglutination in human AB serum was found to be particularly suitable for this purpose.

In the mass investigation of the seed samples the red cells of man, cow, sheep, rabbit, guinea pig, and chicken were used: All the samples were examined by means of human red cells, and approximately two thirds of them by using the other red cells, too. The cells of the animals were tested in saline milieu only.

Agglutination of the human cells was studied also in human AB serum milieu, and usually in kollidon (polyvinylpyrrolidon) milieu and in addition with papain treated cells in saline solution.

The seed extracts were each examined by using the red cells of several people in order that selectively agglutinating specimens might be found. The samples of blood had been chosen so as to be likely to detect a possible ABO, MN, Ss, Pp, or Le^a Le^b

blood group specificity. The animal species were represented by one individual each.

The results seem to indicate that incomplete seed agglutinins, true to the type, are rare, though the kollidon and enzyme treatment methods usually gave higher titres than the saline method. The agglutination by some extracts was clearly promoted by serum, while that by certain others was strongly inhibited by it. The former extracts were often blood group specific, the latter invariably unspecific.

Samples representing approx. 35 % of the 743 species examined by the author proved agglutinative. The occurrence of seed agglutinins in the family Leguminosae seems in some extent to correlate with the taxonomic system of plants. There are genera totally devoid of them and there are others in which the occurrence is as good as the rule. In like manner the agglutinins of the samples of a given genus or species seem to show mutual resemblance as regards their serological properties. There are exceptions to this rule, however.

In general the studied seed agglutinins were species specific to some degree at least. Samples that were found to agglutinate human red cells selectively, represented 69 species. Of these 11 belonged to the group anti-A, 8 to the group anti-A+B, 1 to the group anti-B, 41 to the group anti-H, and 8 to the group anti-N. Part of the anti-H and all of the anti-N extracts were rather weak. Except the anti-N agglutinin of Vicia graminea, the anti-N agglutinins were active only in serum milieu and they are hardly usable in routine work. The anti-A (anti-A_1) of Amphicarpaea species and the anti-B of Bandeiraea simplicifolia may be of use in routine work.

The agglutination-inhibition and elution experiments seem to prove that prosthetic groups of only one kind are usually contained in a given plant extract. They react with a receptor common to the red cells of different animal species. Of the exceptions Vicia cracca extract may be taken as an example. Evidently different prosthetic groups act in the anti-A agglutination on the one hand, and in the anti-man+rabbit+guinea pig on the other. These two groups were nevertheless inseparable being probably parts of one and the same molecule.

A method is suggested to improve the blood group specificity of plant extracts that contain prosthetic groups of more than one kind. In these cases it seems to be possible to saturate some of them with soluble inhibiting substances so that receptors of only one kind remain active.

It seems improbable that the specific receptor of any of the plant agglutinins would be exactly similar to that of an isoagglutinin. According to the author's opinion the red cell receptors reactive with plant agglutinins are generally less complex than those reactive with animal agglutinins.

The results confirm the earlier opinion that in the inhibition of phytagglutination the placement of the substituents in the carbon atoms 3 and 4 of aldoses is of deciding importance.

A substance was found which is secreted in the salivas of both secretors and nonsecretors capable of inhibiting certain seed agglutinins. This substance did not inhibit the agglutination by blood group specific seed extracts. The identity of this substance with Le^a substance is not excluded but seems improbable.

REFERENCES

1. Abel, J. J., and Ford, W. W.: J. biol. Chem. 1907: *2*, 273.
2. Aladjem, F., and Lieberman, M.: J. Immunol. 1952: *69*, 117.
3. Avery, O. T., and Goebel, W. F.: J. exp. Med. 1929: *50*, 533.
4. Bernheimer, A. W., and Farkas, M. E.: J. Immunol. 1953: *70*, 197.
5. Bird, G. W. G.: Curr. Sci. 1951: *20*, 298.
6. Bird, G. W. G.: Indian J. med. Res. 1952: *40*, 585.
7. Bird, G. W. G.: J. Immunol. 1952: *69*, 319.
8. Bird, G. W. G.: Nature 1952: *170*, 674.
9. Bird, G. W. G.: Curr. Sci. 1953: *22*, 273.
10. Bird, G. W. G.: Vox Sanguinis 1953: *3*, 109.
11. Bird, G. W. G.: Acta chir. belg. 1954: Suppl. 7, 33.
12. Bird, G. W. G.: Brit. J. exp. Path. 1954: *35*, 252.
13. Bird, G. W. G.: J. Path. Bact. 1954: *68*, 289.
14. Bird, G. W. G.: Nature 1954: *174*, 1015.
15. Bird, G. W. G.: A. M. C. Journal 1955: 11, 17.
16. Bird, G. W. G.: Nature 1955: *176*, 1127.
17. Bird, G. W. G.: Vox Sanguinis 1956: *1*, (N.S.) 167.
18. Bourdillon, J.: Bull. Soc. Chim. biol. 1949: *31*, 83.
19. Bourdillon, J.: Experientia 1949: *5*, 122.
20. Boyd, W. C., and Reguera, R. M.: J. Immunol. 1949: *62*, 333.
21. Boyd, W. C.: J. Immunol. 1950: *65*, 281.
22. Boyd, W. C.: Fed. Proc. 1952: *11*, 462.
23. Boyd, W. C.: J. Amer. chem. Soc. 1954: *76*, 4026.
24. Boyd, W. C., and Shapleigh, E.: Blood 1954: *9*, 1195.
25. Boyd, W. C., and Shapleigh, E.: J. Immunol. 1954: *73*, 226.
26. Boyd, W. C., and Shapleigh, E.: J. Lab. clin. Med. 1954: *44*, 235.
27. Boyd, W. C., and Shapleigh, E.: Science 1954: *119*, 419.
28. Boyd, W. C.: Rev. Hémat. 1955: *10*, 428.
29. Boyd, W. C., Shapleigh, E., and McMaster, M.: Arch. Biochem. 1955: *55*, 226.
30. Cazal, M., and Lalaurie, M.: Acta haemat. 1952: *8*, 73. (+Appendix).
31. Craeger, W. P., and Gifford, H.: Blood 1952: *7*, 721.
32. Cugudda, E., Gigli, C., and Massenti, S.: Minerva med. 1953: *44*, 140.
33. Danysz, J.: Ann. Inst. Pasteur 1902: *16*, 331.
34. Dufner, I., and Matthes, M.: Arzneimittelforschung 1955: *5*, 36.
35. Dujarric de la Rivière, R., Saint-Paul, M., and Eyquem, A.: C. R. Acad. Sci. (Paris) 1953: *237*, 211.
36. Dujarric de la Rivière, R., Saint-Paul, M., and Eyquem, A.: C. R. Acad. Sci. (Paris) 1955: *240*, 576.
37. Dunsford, I., and Hutchison, A. M.: Vox Sanguinis 1953: *3*, 6.

38. Ehrlich, P.: Dtsch. med. Wschr. 1891, 976 and 1218.
39. Ehrlich, P.: Fortschr. Med. 1897, 41.
40. Ehrlich, P.: Schlussbetrachtungen in Nothnagels Handb. d. spez. Path. u. Ther. 8. (Wien 1901). Cited by H. Sachs and A. Klopstock in Abderhaldens Handb. d. biologischen Arbeitsmethoden, Abt. XIII Teil 2 II p. 840.
41. v. Eisler, M.: Zbl. Bakt., I. Abt. Orig. 1908: *46*, 353.
42. v. Eisler, M., and v. Portheim L.: Z. ImmunForsch. Orig. 1909: *1*, 151.
43. v. Eisler, M., and v. Portheim L.: Zbl. Bakt., I. Abt. Orig. 1912: *66*, 309.
44. v. Eisler, M., and v. Portheim L.: Z. ImmunForsch. 1926: *47*, 59.
45. Elo, J., Estola, E., and Malmström, N.: Ann. Med. exp. Biol. Fenn. 1951: *29*, 297.
46. Elo, J., and Estola, E.: Ann. Med. exp. Biol. Fenn. 1952: *30*, 165.
47. Estola, E., and Vartia, K. O.: Ann. Med. exp. Biol. Fenn. 1955: *33*, 392.
48. Eyquem, A., Saint-Paul, M., and Magnin, J.: Ann. Inst. Pasteur 1955: *89*, 60.
49. Ford, W. W.: Zbl. Bakt., I. Abt. Ref. 1913: *58*, 129 and 193.
50. Friedberger, E., and Brossa, G. A.: Z. ImmunForsch. 1912: *15*, 506.
51. Friedemann, U.: Z. ImmunForsch. Orig. 1909: *2*, 591.
52. Friedenreich, V., and With, S.: Z. ImmunForsch. 1933: *78*, 152.
53. Fujiwara, K.: Biochem. Z. 1923: *140*, 113.
54. Furihata, R.: J. Biochem. (Tokyo) 1951: *38*, 361.
55. Galli-Valerio, B., and Bornard, M.: Z. ImmunForsch. 1916: *25*, 154.
56. Herrmann, W.: Discussion note to the article of M. Krüpe: Zbl. Bakt., I. Abt. Orig. 1953: *160*, 289.
57. Hummel, K.: Zbl. Bakt., I. Abt. Ref. 1955: *155*, 1.
58. Isotalo, S.: Ann. Med. exp. Biol. Fenn. 1950: *28*, 187.
59. Jacoby, M.: Beitr. z. chem. Physiol. u. Pathol. 1904: *4*, 213. Cited by W. W. Ford Zbl. Bakt., I. Abt. Ref. 1913: *58*, 193.
60. Jacoby, M.: Handb. d. pathogenen Mikroorganismen Kolle-Wassermann 3. Aufl. Bd III $_1$: 1930, p. 107.
61. Kabat, E. A., and Leskowitz, S.: Fed. Proc. 1955: *14*, 467.
62. Kabat, E. A., and Leskowitz, S.: J. Amer. chem. Soc. 1955: *77*, 5159.
63. Kabat, E. A.: Blood Group Substances. Their Chemistry and Immunochemistry. Academic Press Inc., New York 1956.
64. Kahl, L.: Arzneimittelforschung 1955: *5*, 34.
65. Kirchner, M.: Über Vorkommen und Art der Anti-A-spezifischen Haemagglutinine in Limabohnen (Phaseolus limensis). Inaug. Diss. Marburg/Lahn 1952.
66. Kobert, R.: Arch. d. Vereins d. Freunde d. Naturgesch. in Mecklenburg (Anhang) 1900, p. XIII.
67. Kobert, R.: Lehrbuch der Intoxicationen. 2 Aufl. Ferdinand Enke Verlag, Stuttgart 1906, pp. 161 and 695.
68. Kossovitch, N., and Canat, J.: C.R. Soc. Biol. (Paris) 1941: *135*, 1100.

69. Koulumies, R.: Ann. Med. exp. Biol. Fenn. 1949: *27*, 20.
70. Koulumies, R.: Ann. Med. exp. Biol. Fenn. 1949: *27*, 185.
71. Koulumies, R.: Ann. Med. exp. Biol. Fenn. 1950: *28*, 160.
72. Kraus, R.: Z. Heilkunde 1902: *23*, 369.
73. Kraus, R., and Ludwig, S.: Wien. klin. Wschr. 1902, 120.
74. Kritschewsky, J. L.: Z. ImmunForsch. 1914: *22*, 381.
75. Krüpe, M.: Z. ImmunForsch. 1950: *107*, 450.
76. Krüpe, M., and Powilleit, E.: Z. Hyg. InfektKr. 1952: *134*, 198.
77. Krüpe, M., and Braun, C.: Naturwissenschaften 1952: *39*, 284.
78. Krüpe, M.: Zbl. Bakt., I. Abt. Orig. 1953: *160*, 289.
79. Krüpe, M.: Biol. Zbl. 1953: *72*, 424.
80. Krüpe, M.: Z. Hyg. InfektKr. 1953: *138*, 167.
81. Krüpe, M.: Paper no. 356. Sixth International Congress for Microbiology 1953.
82. Krüpe, M.: Z. ImmunForsch. 1954: *111*, 22.
83. Krüpe, M.: Hoppe-Seyl. Z. physiol. Chem. 1955: *299*, 277.
84. Krüpe, M.: Blutgruppenspezifische pflanzliche Eiweisskörper (Phytagglutinine). Ferdinand Enke Verlag, Stuttgart 1956.
85. Krüpe, M., and Dödzer, W.: Ann. Med. exp. Biol. Fenn. 1957 (in press).
86. Kuhns, W. J., and Bailey, A.: Amer. J. clin. Path. 1950: *20*, 1067.
87. Landsteiner, K.: Wien. klin. Rdsch. 1902, 774.
88. Landsteiner, K., and Stankovic, R.: Zbl. Bakt., I. Abt. Orig. 1906: *41*, 108.
89. Landsteiner, K., and Raubitschek, H.: Zbl. Bakt., I. Abt. Orig. 1908: *45*, 660.
90. Landsteiner, K., and Raubitschek, R.: Biochem. Z. 1909: *15*, 33.
91. Landsteiner, K., and Miller, C.P.: J. exp. Med. 1925: *42*, 853.
92. Landsteiner, K.: The Specificity of Serological Reactions. Harvard University Press, Cambridge, U.S.A. 1947.
93. Lau, C.: Über vegetabilische Blutagglutinine. Inaug. Diss., Rostock 1901.
94. Levine, P., Ottensooser, F., Celano, M. J., and Pollitzer, W.: Amer. J. phys. Anthrop. 1955: *13*, 29.
95. Li, J. G., and Osgood, E. E.: Blood 1949: *4*, 670.
96. Liener, I. E., and Wada, S.: J. biol. Chem. 1956: *222*, 695.
97. Lust, F. A.: Beitr. z. chem. Physiol. u. Pathol. 1905: *6*, 132. Cited by W. W. Ford Zbl. Bakt., I. Abt. Ref. 1913: *58*, 193.
98. di Macco, G.: Z. ImmunForsch. 1923: *38*, 467.
99. Mäkelä, O., and Mäkelä, P.: Ann. Med. exp. Biol. Fenn. 1956: *34*, 402.
100. Mäkelä, O., Mäkelä, P., and Linkola, H.: To be published.
101. Mäkelä, O.: Unpublished observations.
102. Marchal, J-G.: C.R. Soc. Biol. (Paris) 1942: *136*, 760.
103. Marcusson-Begun, H.: Z. ImmunForsch. 1926: *45*, 49.
104. McNeil, C., Trentelman, E. F., Kreutzer, V., and Fullmer, C. D.: Amer. J. clin. Path. 1955: *25*, 1205.
105. Miessner, and Rewald: Z. ImmunForsch. Orig. 1909: *2*, 323.
106. Mohn, J. F.: Sixth Congress of the International Society of Blood Transfusion, Boston, 1956.

107. Morgan, W. T. J.: Proc. roy. Soc. Med. 1953: *46*, 783.
108. Morgan, W. T. J., and Watkins, W. M.: Brit. J. exp. Path. 1953: *34*, 94.
109. Müller, F.: Arch. exp. Path. Pharmak. 1899: *42*, 302.
110. Munter, J.: Ann. Med. exp. Biol. Fenn. 1949: *27*, 12.
111. Neter, E.: Bact. Rev. 1956: *20*, 166.
112. Nungester, W. J., and van Halsema, G.: Proc. Soc. exp. Biol. 1953: *83*, 863.
113. Ottensooser, F., and Lenzinger, A.: Z. ImmunForsch. 1933: *81*, 354.
114. Ottensooser, F., and Silberschmidt, K.: Nature 1953: *172*, 914.
115. Ottensooser, F.: Arch. Biol. (S.Paulo) 1955: *39*, 76.
116. Ottensooser, F.: Anais da Academica Brasilieira de Ciencias 1955: *27*, 519.
117. Porges, O.: Zbl. Bakt., I. Abt. Orig. 1905: *40*, 133.
118. Punin, W.: Z. Naturf. 1952: *7b*, 48.
119. Raubitschek, H.: Wien. klin. Wschr. 1909, 1752.
120. Raubitschek, H., and Wilenko, M.: Z. ImmunForsch. Orig. 1910: *5*, 446.
121. Raubitschek, H.: Z. ImmunForsch. Orig. 1911: *9*, 297.
122. Renkonen, K. O.: Ann. Med. exp. Biol. Fenn. 1948: *26*, 66.
123. Renkonen, K. O.: Ann. Med. exp. Biol. Fenn. 1950: *28*, 45.
124. Renkonen, K. O., and Therman, E.: Ann. Med. exp. Biol. Fenn.: 1952: *30*, 327.
125. Rigas, D. A., and Osgood, E. F.: J. biol. Chem. 1955: *212*, 607.
126. Römer, P.: Archiv f. Ophthal. 1901: *52*, 72.
127. Rona, P., and György, P.: Biochem. Z. 1920: *105*, 121.
128. Rona, P., and Krebs, H. A.: Biochem. Z. 1926: *169*, 266.
129. Rosenthal, L.: J. Bact. 1943: *45*, 545.
130. Russ, V. K., and Oesterlin, E.: Biochem. Z. 1921: *114*, 258.
131. Schmidt, G.: Z. ImmunForsch. 1954: *111*, 432.
132. Schmidt, H.: Discussion note to the article of L. Huber: Schweiz. Z. allg. Path. Bact. 1953: *16*, 789.
133. Sievers, O.: Studier över isoagglutinationen. Inaug. Diss., Helsinki 1927, p. 16.
134. Skoog, W. A., and Beck, W. S.: Blood 1956: *11*, 436.
135. Speiser, P., Baumgarten, K., and Kaserer, O.: Z. ImmunForsch. 1954: *111*, 168.
136. Springer, G. F.: Naturwissenschaften 1955: *42*, 37.
137. Springer, G. F.: Naturwissenschaften 1956: *43*, 256.
138. Springer, G. F.: J. Immunol. 1956: *76*, 399.
139. Stillmark, H.: Über Ricin, ein giftiges Ferment aus den Samen von Ricinus comm. L. und einigen anderen Euphorbiaceen. Inaug. Diss., Dorpat 1888.
140. Sumner, J. B., and Howell, S. F.: J. Bact. 1936: *32*, 227.
141. Tétry, A., Sutton, É., and Moullec, J.: C. R. Acad. Sci. (Paris) 1953: *237*, 1566.
142. Tétry, A., Sutton, É., and Moullec, J.: C. R. Acad. Sci. (Paris) 1954: *238*, 277.

143. Tétry, A., Sutton, É., and Moullec, J.: C. R. Acad. Sci. (Paris) 1955: *240,* 2434.
144. Tiggelman-van Krugten, W. A. H., Ostendorf-Doyer, C. M., and Collier, V. A.: Antonie v. Leeuwenhoek 1956: *22,* 289.
145. Watkins, W. M., and Morgan, W. T. J.: Nature 1952: *169,* 825.
146. Wiener, A. S., and Wexler, I. B.: Bact. Rev. 1952: *16,* 69.
147. Wienhaus, O.: Biochem. Z. 1909: *18,* 228.
148. Wilenko, M.: Z. ImmunForsch. Orig. 1910: *5,* 91.
149. Willis, J. C.: A Dictionary of the Flowering Plants and Ferns. Cambridge University Press, Cambridge 1951.
150. Yamakami, K.: J. Immunol. 1926: *12,* 185.